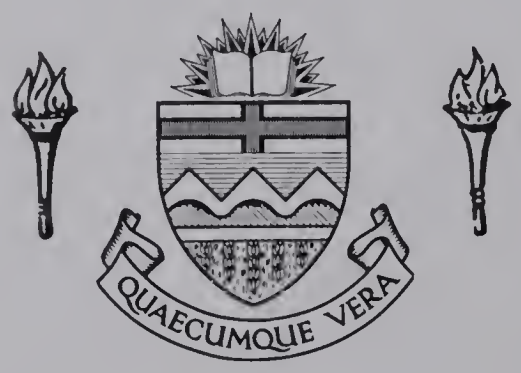

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SUPPLEMENTARY COPPER FOR SWINE

by

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A THESIS

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled "Supplementary Copper for Swine" submitted by Adrian George Castell, M.Sc., in partial fulfilment of the requirements for the degree of Doctor of Philsophy.

ABSTRACT

Four experiments were conducted during 1963 to 1966 to study the effects of inclusion of 0.1% of copper sulfate in diets of crossbred pigs from weaning to market weight.

Experiment 1 involved 64 pigs group fed, ad libitum, diets which varied in supplemental protein source, fishmeal or soybean meal, and crude protein content, 14 or 17 per cent. The effects of removal of supplementary copper and reduction of protein content after pigs reached an average weight of 57 kg. were compared to the responses of pigs fed a constant level of protein and copper over the entire growing period.

In Experiment 2, 48 pigs were allocated to fishmeal based diets fed ad libitum. The treatments differed in level of supplementary protein, 7.5 or 2.5%, which was reflected in the dietary protein contents, 18.0 and 13.0% respectively. Supplementary copper was added factorially to each diet at each protein level.

Experiments 3 and 4, involving 8 and 24 pigs, respectively, yielded data on copper supplementation of diets based on fishmeal when the dietary intakes were restricted to a scale based on individual liveweight.

The criteria measured in all experiments were average daily feed intake (A.D.F), average daily gain (A.D.G.) and efficiency of feed conversion (F.C.E.). Blood hemoglobin values were obtained at different stages of growth in Experiments 1, 2 and 4. Blood serum measurements included serum copper and total protein levels and relative amounts of serum protein fractions in Experiments 3 and 4. In addition, tissue copper content of liver, kidney, heart, spleen, muscle and hair were

in energy and protein utilization. During the rearing period for copper supplemented pigs, both apparent digestibility of energy and of nitrogen were increased.

The beneficial effects of copper in the reported experiments were limited to the early portion of the growth period. It has been suggested that its major effect at that time might be to offset an apparent temporary copper deficiency brought about by insufficient assimilation of copper from the diet prior to and following weaning.

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INTRODUCTION

Sufficient evidence has accumulated to establish that copper is an essential element for the normal growth of higher plants and of animals. In practice, animal diets, based on usual feedstuffs, rarely contain less than five parts per million (p.p.m.) of copper, which should meet requirements. Therefore, under normal conditions the diets of domestic animals and humans after weaning contain adequate amounts of copper and supplements are not only unnecessary but may result in toxicity. The pig appears to be an exception in that supplementation of swine diets with copper compounds has in many cases resulted in beneficial effects. Copper sulfate is widely used in Europe as a feed additive to promote increased rate of gain but has not been generally accepted in North America.

LITERATURE REVIEW

Initial Observations

The earliest report that supplementation of diets with copper sulfate improved the growth rate in pigs was that of Evvard, Nelson and Sewell (1928), but little interest was apparent until the observations by Braude (1945) that pigs under certain conditions exhibited an obvious craving for metallic copper. In the same report it was noted that while the pigs showed a desire to consume additional copper, no significant effects were observed when a basal ration, containing 8.5 p.p.m. copper was supplemented with 25 or 50 mg. copper per pig per day.

It was thought that the addition of copper might stimulate feed intake. With a view to increasing the feed consumption of suckling pigs the effects of adding copper sulfate, at a level equivalent to 150 p.p.m., were tested by Mitchell (1953). Acceptibility of the ration when copper was included was increased markedly. However a later report, using a larger number of litters, did not confirm this result (Barber, Braude and Mitchell, 1955a).

General Responses to Added Copper

Evidence for the growth promoting property of copper was presented by Barber et al. (1955). They studied the effects of a commercial mineral mixture XF* containing 4% copper sulfate, added at 2.5% (equivalent to 250 p.p.m. of Cu) to a ration fed to scale to weanling pigs for a period of eighteen weeks. While there were no statistical differences in weight gains, feed consumption, feed conversion efficiency or carcass grading, it was observed that during the first eight weeks, rate of gain, feed consumption and efficiency of feed utilization were improved for

*Minsal Ltd., Northwich, England.

those pigs receiving the supplements. Repeating the experiment in a large scale field trial in England, where the rations and feeding practices for the individual centres were maintained, there was an overall statistically significant improvement in growth rate and a non-significant benefit in feed conversion efficiency when the mineral mixture was included in the diets (Bowler et al., 1955).

Numerous experiments have since been conducted to estimate the effect of supplementary copper on the two main criteria of assessing the performance of pigs, i.e. daily liveweight gain (d.l.w.g.) and feed conversion efficiency (f.c.e.). Braude (1965) analyzing the results of 83 experiments found that the average improvement was +8.1% for d.l.w.g. and +5.4% for f.c.e. when copper was supplemented at a level equivalent to 250 p.p.m.

The effects of supplementary copper on the carcass quality of pigs is not clear. Some investigators have found the backfat thickness to be increased in pigs receiving high levels of copper (Barber et al., 1957; Barber, Braude and Mitchell, 1960; Allen et al., 1961). Increases in the dressing percentage (Lucas and Calder, 1957a; Allen et al., 1958; Barber, Braude and Mitchell, 1960; Barber et al., 1961; Allen et al., 1961) and reduction in carcass length (Barber, Braude and Mitchell, 1960; Barber et al., 1961; Allen et al., 1961) have also been observed. However, in the majority of reports there has been no apparent effect on carcass measurements.

Responses to Copper and Antibiotics

The similarity in the effects of adding dietary copper to the responses obtained when antibiotic was included in swine diets was apparent early in the investigations with copper supplements. Barber,

Braude and Mitchell (1955b) studied the effects of including 2.5% mineral mix XF, 0.125% Aurofac**2A, Aurofac plus XF or 0.1% $CuSO_4.5H_2O$[1] in a diet individually fed to ten-week old weanling pigs. All the supplements increased the rate of liveweight gain, the first three notably during the first eight weeks of the experiment but only the copper sulfate supplement showed an advantage over the entire growing period and a significant improvement in f.c.e. In a later experiment which included other antibiotics in addition to the forementioned supplements it was confirmed that 0.1% copper sulfate could significantly increase growth rate as well as feed intake (Barber et al., 1957).

Experiments comparing the value of antibiotics and copper salts as supplements present conflicting data. In some reports similar responses occur (Barber et al., 1957; Hawbaker et al., 1959; Bunch et al., 1961), in some tests antibiotics were superior (Wallace et al., 1960; Beames and Lloyd, 1965) but the majority of experiments favor copper supplementation (Lucas and Calder, 1957b; Barber et al., 1957; Barber, Braude and Mitchell, 1960; Bellis, 1961; Braude et al., 1962). Several workers have suggested that the effect of joint supplementation is additive under certain experimental conditions (Lucas and Calder, 1957b; Hawbaker et al., 1959; Lucas et al., 1962) while this has not been confirmed in other reports (Wallace et al., 1960; Braude et al., 1962).

The apparent similarity of results when copper or antibiotic was added to swine rations stimulated several workers to study the effects

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**Lederle Laboratories Inc. containing 7.9 gm. chlortetracycline/kg.

[1] = 250 p.p.m. Cu

of supplemental copper on the flora of the digestive tract. There was an early indication that copper included in the diet could significantly change the fecal flora pattern (Hawbaker et al., 1959). In a more extensive report evidence was presented that inclusion of 0.1% copper sulfate significantly increased numbers of coliform bacteria, molds and yeasts and decreased lactobacilli, aerobes, anaerobes and streptococci (Hawbaker et al., 1961). The results were similar to those obtained when antibiotics were included in the diet.

Support for the hypothesis that copper may be effective on account of its action on the intestinal flora is not conclusive (Fuller et al., 1960; Bunch et al., 1961; Williams-Smith and Jones, 1963). It was suggested that it is doubtful that the mode of action of copper is a result of its fungicidal or anthelminthic properties (Braude, 1965).

Effect of Form and Level of Copper on Responses

The original observations by Braude (1945) suggested that the copper ion was responsible for the beneficial effects but as most subsequent experiments utilized copper sulfate it was necessary to confirm that copper was involved.

Hawbaker and co-workers (1959) reported that a supplement of copper chloride effectively improved average daily gain and feed conversion efficiency while an equivalent amount of sulfate, as the sodium salt, did not. Using microanalytical grade $CuSO_4.5H_2O$ it was confirmed that copper sulfate rather than some impurity in the commercial grade was responsible for the benefits obtained (Lucas, Livingstone and McDonald, 1961).

Data have been accumulated indicating that soluble copper salts other than the sulfate or chloride can serve as effective sources of supplementary

copper, notably copper carbonate (Allen et al., 1961; Bunch et al., 1965) and copper oxide (Bunch et al., 1961; Bunch et al., 1963). Using isotopically labelled copper compounds it was reported that copper availability measured by the concentration of Cu^{64} in the blood of swine was approximately the same whether the sulfate, oxide or carbonate was used as the source (Buescher, Griffin and Bell, 1961).

Copper sulfide, a highly insoluble copper salt, has been reported to have little if any effect when included in swine rations at a level equivalent to 250 p.p.m. Cu (Barber et al., 1960; 1961). Using $Cu^{64}S$ an average of 1.7% of the dose was absorbed compared to 5.1% when $Cu^{64}SO_4$ was given orally (Bowland et al., 1961). The mode of action of copper appears to be related to the extent of its solubility in the intestinal tract.

Numerous experiments have been conducted in an attempt to assess the optimum level of copper supplementation. The most widely accepted rate of inclusion in diets from weaning to market weight is 0.1% copper sulfate, equivalent to a copper level of approximately 250 p.p.m. of diet (Braude, 1965). Inclusion of copper at levels greater than 500 p.p.m. presents a danger of toxicity and shows minimal if any benefits over unsupplemented diets. Supplementation equivalent to 250 p.p.m. Cu and lower rates has occasionally produced adverse effects and apparent symptoms of copper toxicity (Bass et al., 1956; Wallace et al., 1960; Buntain, 1961).

Responses to Copper at Different Stages of Growth

Copper supplementation of rations fed from any period from two weeks of age to market weight has been reported to be beneficial. A ration containing 0.1% copper sulfate had no adverse effect when

introduced immediately after weaning at approximately 3.6 kg. liveweight. Up to 20.4 kg. liveweight the supplemented diet resulted in improvements of 6 and 12% in daily liveweight gain and 5 and 6% in feed conversion efficiency respectively in two experiments conducted by Lucas et al., (1962). Similar results were obtained using an equivalent level of copper carbonate (Bunch et al., 1965).

It is a matter for conjecture whether copper supplementation is of value over the entire growing period. Early observations by Bowland (1954), and Barber et al. (1955), suggested that the greatest response occurred early in the growing period. Later experiments presented supporting evidence (Lucas, Livingstone and McDonald, 1961: Lucas, Livingstone and Boyne, 1962; Wallace et al., 1962; Anastasijevic, Braude and Rowell, 1963) but other workers have stressed the advantage of including copper over the entire growing period (Barber, Braude and Mitchell, 1955b; Lucas and Calder, 1957b; Fagan et al., 1961; Bellis, 1961; Leroch, 1963).

One report by Lucas and Calder (1957a) indicated that lowering the level of added copper sulfate during the final stage of growth resulted in optimal responses in liveweight gain over the entire period.

The effect of supplementation of sow rations has been virtually ignored although long term feeding of 2.3 gm. copper sulfate per day to sows showed no adverse or beneficial effects (Dammers and van der Grift, 1959).

The comparative responses of different breeds to dietary copper supplements have not been reported.

Effects of Ration Composition

A) Type and level of protein.

It has been observed that consistent responses to copper supplements occur in Europe while experiments in North American yield variable results. Swine rations in North America are usually based on cereals, notably corn, supplemented with vegetable protein, often soybean meal. In European rations the cereal protion rarely contains much corn while the protein supplement frequently consists of fish or meat meal. Furthermore, American rations are usually fed ad libitum and tend to be lower in crude protein and higher in energy than comparable European rations which are usually fed to scale at least in the final stages of growth.

Several workers have conducted experiments to study the responses to supplemental copper in diets containing a variety of protein sources. Lucas, Livingstone and Boyne (1962) studied the effects of inclusion of 0.1% copper sulfate in each of three diets. Two of the diets contained the same level of protein but were based on either barley and fishmeal or corn and soybean meal, the third was a corn-soybean meal of lower protein content. All diets were fed to scale and were reduced in protein content when pigs reached an average of 47 kg. liveweight. The copper significantly increased the rate of gain by 7 - 8% on all diets before 47 kg. liveweight. Between 47 kg. and market weight it did not affect performance of pigs on the higher protein levels but reduced rate of gain and the efficiency of feed conversion of the pigs fed the lower level of protein. Copper improved the f.c.e. only in pigs fed the barley-

fish meal diet.

In a similar experiment Barber, Braude and Mitchell (1962) reported that the same level of copper added to diets based on barley and fishmeal or barley and soybean meal resulted in a greater response in the pigs fed the animal protein supplemented diet.

No significant differences in d.l.w.g. or f.c.e. were reported when 250 p.p.m. Cu was included in diets containing mainly barley meal and one of the following protein sources: - 10% white fish meal, 15.75% soybean meal, 24% dried skimmilk or 10% soybean meal plus 5.25% meat and bone meal. All diets were supplemented with minerals and vitamins and were fed to scale over a ten week period (O'Donovan, Spillane and O'Grady, 1966).

Other reports (Beames and Lloyd, 1965; Combs et al., 1966) comparing the response to copper with specific protein sources failed to confirm that the action of copper is dependent upon a particular form of diet.

The effects of level of protein in the diet on response to copper have been studied but with variable results (Bunch et al., 1961; Lucas, Livingstone and Boyne, 1962; King, 1964).

B) Mineral composition of the diet.

The copper requirement of swine is reported to be 10 p.p.m. in Nutritional Requirements of Swine (N.R.C. 1964), but this requirement is based on studies with baby pigs. Other reports in the literature present a similar recommendation (Braude, 1965). Normal rations can be assumed to contain at least 5 p.p.m. Cu. As far as can be ascertained from published reports the response to copper supplementation has occurred with diets adequately supplied with necessary minerals

and vitamins. Consequently, it would appear unlikely that mineral interrelationships play an important role in the mode of action of supplemental copper.

Copper is involved in several interrelationships with other minerals. In swine, copper levels have been studied in conjunction with levels of molybdenum (Kulwich et al., 1953), zinc (Hoefer et al., 1960; Allen et al., 1958; Barber, Braude and Mitchell, 1960), iron (Hoefer et al., 1960; Ritchie et al., 1963) and calcium (Carter, Miller and Brooks, 1959).

It has been suggested by Kirchgessner and Weser (1963) that supplemental copper could displace other heavy metals from complexes with proteins, peptides or amino acids and thus promote their intestinal absorption.

Forms of Copper Normally Present in Swine

The whole body copper concentration on a fat-free basis has been reported to be 3.2 p.p.m. and 2.5 p.p.m. for the newborn and adult pig, respectively (Spray and Widdowson, 1950; Widdowson, 1950). The higher value for copper in newborn pigs is partially a reflection of the higher concentration of liver copper which decreases throughout the growing period although the total content of copper in the liver increases. Cunningham (1931) reported levels of liver copper in newborn and adult pigs of 233 p.p.m. dry matter (total 2.0 mg.) and 41.3 p.p.m. dry matter (total 19.9 mg.) respectively.

The distribution of copper within the body is fairly consistent although published values for tissue copper concentrations are highly variable. In general, the endocrine glands (pituitary, thyroid and thymus) are examples of low copper tissues and the liver, heart, kidneys,

hair and brain, usually in that order, are examples of tissues with a high concentration of copper. The spleen and bones represent organs of intermediate copper concentration (Underwood, 1962).

The liver serves as the main storage organ for copper in the body and as such is subject to greater variation in copper concentration than most other tissues. Even in animals maintained upon the same dietary regimen,large differences in liver copper levels have been observed. To a lesser extent the kidney copper levels are subject to fluctuations but under normal conditions the copper concentration in other organs is relatively constant (Underwood, 1962).

Several copper complexes have been isolated from animals since the identification of two copper containing proteins, hemocuprein from red blood cells and hepatocuprein from the liver of cattle (Mann and Keilin, 1938). In 1960, Scheinberg and Sternlieb (1960) summarized the characteristics of a further seven mammalian copper proteins: erythrocuprein obtained from human erythrocytes, cerebrocuprein from human brain tissue, horse liver copper protein, ceruloplasmin from human plasma, cytochrome C oxidase from beef heart tissue, tyrosinase from mouse melanoma and uricase from hog liver. Enzyme activity in the last four complexes has been demonstrated.

Subsequently other copper proteins have been reported. Yamada et al., (1963) presented evidence that plasma amine oxidase isolated from beef plasma contained copper at the active site of the enzyme. Neonatal hepatic mitochondro-cupreins obtained from immature bovine liver and newborn human liver were found to contain $>$ 4% and $>$ 2% copper respectively (Porter,Johnston and Porter, 1962; Porter, Sweeney and Porter, 1964).

Apart from the above mentioned naturally occurring complexes of

copper it undoubtedly exists to some extent in vivo in other forms. These would include free cupric or cuprous ions and combinations of copper ions with amino acids, purines, pyrimidines, nucleotides, DNA, RNA and proteins (Klotz and Curme, 1948; Fiess and Klotz, 1952; Frieden and Alles, 1958; Scaife, 1959a, 1959b; Wacker and Vallee, 1959).

Metabolism of Copper

A) Absorption

There is limited information on the mechanism of absorption of copper. In pigs the main site of absorption was reported to be the small intestine and colon by Bowland et al., (1961). Studies have shown that the amount absorbed can be profoundly affected by other dietary factors and the chemical form, level and combinations of copper ingested. Using the changes in copper concentration in the liver or blood as a measure of relative absorption it has been reported that type and level of protein in the diet (Beames and Lloyd, 1965; Combs et al., 1966; O'Donovan, Spillane and O'Grady, 1966), levels of other minerals, notably molybdenum, iron, zinc and calcium, (Kulwich et al., 1953; Allen et al., 1958; Miller et al., 1959; Barber, Braude and Mitchell, 1960; Suttle and Mills, 1964), the level of copper (Bass et al., 1956; Lucas and Calder, 1957a; Ullrey et al., 1960; Allen et al., 1961) or form of copper (Barber et al., 1960; Buescher, Griffin and Bell, 1961; Bunch et al., 1965) can influence the assimilation of copper from the diet. Even under optimum conditions only 2-10% of the ingested copper is absorbed and retained by the pig (Bowland et al., 1961).

There is also limited information upon the form of naturally occurring copper in feedstuffs. However, Mills (1956) reported that

an aqueous extract of herbage contained a source of available copper which proved superior to copper sulfate for administration to copper deficient rats.

B) Blood copper.

After absorption, copper is transported in the blood plasma loosely bound to a plasma protein, probably albumin (Underwood, 1962). In the pig and other species the blood copper can be separated into erythrocyte copper and plasma copper, the latter can be further apportioned between the direct-reacting fraction (the copper which reacts directly with aqueous sodium diethyldithiocarbamate) and the indirect-reacting fraction. It is generally accepted that the direct reacting fraction represents copper transported in the albumin portion of plasma. The indirect-reacting copper is mainly, if not all,the copper in ceruloplasmin, an α-globulin with a content of 0.34% copper.

Comparing the blood copper picture in human beings, rats, dogs and pigs, Gubler and co-workers (1953) reported that in the pig the indirect-reacting copper constituted only about 58% of the total copper in plasma, compared to 88%, 99% and 96% in the dog, rat and human being, respectively. In addition, the rate at which copper in the direct-reacting fraction combined with carbamate was faster in pig plasma than in the other three species.

The erythrocyte and indirect-reacting fractions of blood initially maintain a relatively unchanged copper content when copper is administered orally or intravenously (Gubler et al., 1953). The direct reacting fraction increases to an extent which has been correlated with the amount of copper absorbed. Over a long period of administration the level of ceruloplasmin in blood was elevated

with the increase in serum copper levels (Bunch et al., 1965).

In human beings the concentration of ceruloplasmin and copper in serum has been found to rise significantly with infections, in several organic diseases, in the latter part of pregnancy and after administration of certain hormones (Scheinberg and Sternlieb, 1960). In pigs ceruloplasmin level appeared to be elevated to a greater extent by stilbestrol than by estrone administration but a subsequent trial could not confirm these results (Bunch et al., 1965). The effects of disease and other physiological factors on serum copper and ceruloplasmin levels in swine have not been reported.

In vitro studies with human ceruloplasmin incubated with Cu^{64}, buffer and sufficient ascorbic acid to maintain the ceruloplasmic copper in a cuprous form suggest that at least half of the copper in the ceruloplasmin molecule is exchangeable (Morell and Scheinberg, 1958; Curzon, 1959). Similar studies with human red blood cells, using Cu^{64}, in vitro and in vivo have demonstrated an exchange of erythrocyte copper with plasma copper, favouring uptake from the plasma (Bush et al., 1956a). Comparative studies in swine have not been reported.

From the blood, absorbed copper is widely distributed to the tissues but appears to accumulate mainly in the parenchymal cells of the liver and kidney. In the liver the copper may be stored, incorporated into the various copper containing enzymes of the cells and such compounds as hemocuprein and ceruloplasmin or secreted into the bile and excreted via this route back to the intestinal contents (Underwood, 1962).

C) Excretion of copper.

The major pathway of copper excretion in swine is the feces. Much of the fecal copper represents copper that has not been absorbed from the ration although Bowland et al., (1961) have calculated that the bile could account for up to 40% of the total amount excreted in the feces. Pigs under normal conditions excrete only small quantities of copper via the kidneys or intestinal wall (Mahoney et al., 1955). Urinary copper was found to significantly increase where the source fed was copper carbonate rather than the oxide or sulfate, while fecal excretion showed no significant differences (Buescher, Griffin and Bell, 1961).

D) Metabolic role of copper.

Much information concerning the various roles of copper in animals has been obtained by examining the changes which occur in copper deficiency. Natural copper deficiency can occur in young animals as a result of feeding low copper diets such as milk. Under such conditions the reserves of copper, mainly liver copper, are rapidly depleted. Similarly copper deficiency may arise by feeding rations derived from copper deficient areas or containing high levels of elements which limit the availability of the dietary copper.

1) Copper and hematopoiesis

Piglets consuming a low copper diet ultimately develop an anemia classified as microcytic and hypochromic and which is morphologically identical to the anemia of iron deficiency (Cartwright et al., 1956). Utilizing ferrokinetic studies and isotopic iron and copper, Bush and co-workers (1956b) reported that the numbers of erythrocytes produced and their average life span in copper deficient swine were

markedly reduced. The life span decreased to an average of 13 days compared to a normal value of 63 days. The authors suggest that a certain minimum level of copper must be available for the production of red corpuscles and to maintain their integrity in the circulation.

Many experiments have been conducted with swine and other species in an attempt to identify the exact role of copper in hematopoiesis but the evidence is inconclusive.

2) Copper and iron metabolism

Copper deficiency in swine has been reported to result in an impaired ability to absorb iron from the intestinal tract although transport and mobilization of iron from the tissues does not appear to be directly affected (Bush et al., 1956b). Matrone (1960) in reviewing the interrelationships of iron and copper suggests that iron tissue deposition, release and transport in copper deficient swine can be explained on the basis of diminished erythrocyte production.

3) Copper and bone formation

In addition to dietary anemia, copper deficiency has resulted in certain bone abnormalities in swine. Ad libitum feeding of a whole milk diet to young pigs led to a progressive loss of use of the hind, then fore legs (Teague and Carpenter, 1951). Supplemental copper at a rate of 2 mg./pig/day appeared capable of arresting the development of the symptoms. Administration of copper and iron resulted in a degree of reversal in the degenerative process but supplementing with vitamins A and D had no effect. Similar observations were reported by Lahey et al., (1952) but a smaller proportion of deficient animals developed the symptoms, possibly as a result of a

higher level of dietary iron.

Investigations of the effects of low levels of dietary copper revealed a marked diminution of osteoblastic activity in bone sections from deficient pigs (Follis et al., 1955). The authors remarked on the similarity of the symptoms to those of scurvy and reported that both copper and ascorbic acid seem to have a unique property in common, i.e. ability to interfere specifically with the functional activity of the osteoblasts while not affecting the integrity of cartilage cells.

The mechanism of copper in the process of bone formation has not been identified.

4) Copper in other physiological processes

In species other than swine experimental results have suggested that copper is involved in phospholipid synthesis (Gallagher et al., 1956b), keratin and pigment formation (Underwood, 1962) and reproductive processes (Allcroft and Parker, 1949).

The Role of Copper Proteins

a) Ceruloplasmin

In vitro, ceruloplasmin exhibits oxidase activity toward certain polyphenols and polyamines. The best substrate appears to be para-phenylenediamine (PPD) in which the maximum activity of the enzyme lies between pH 5 and 6 (Holmberg and Laurell, 1951a). The activity is dependent upon the ceruloplasmic copper and can be affected by the presence of different anions (Holmberg and Laurell, 1951b; 1951c). A physiological significance for the oxidase activity of ceruloplasmin has not been established in vivo and the role of ceruloplasmin is

unknown.

In humans a deficiency, or absence of ceruloplasmin occurs in almost all patients afflicted with hepatolenticular degeneration (Wilson's Disease).

B) Cytochrome C oxidase

As the terminal member of the mitochondrial electron transport chain, cytochrome C oxidase is the only member capable of reducing oxygen. The purified enzyme is a polymer of subunits of molecular weight of about 72,000, each of which contains one heme molecule as well as one atom of copper. The function of copper appears to be intimately connected with the action of the enzyme. When the enzyme is reduced by a substrate the copper is reduced to a cuprous form (Griffiths and Beinert, 1961). In swine, deficiency of copper markedly decreased the cytochrome oxidase activity in the heart and liver (Gubler, Cartwright and Wintrobe, 1957).

c) Uricase

Porcine hepatic uricase which catalyzes the oxidation of uric acid to allantoin possesses enzymatic activity which appears to be related to the presence of copper in the enzyme (Mahler, Hubscher and Baun, 1955). No information concerning the effect of copper deficiency on uricase levels has been reported.

D) Tyrosinase

Tyrosinase shows considerable chemical resemblance to ceruloplasmin. It exhibits oxidase activity toward poly and monophenols, reversibility of the copper protein bond and an inherited deficiency occurs (Scheinberg and Sternlieb, 1960). It is involved in melanin formation,

using tyrosine and dihydroxyphenylalanine (DOPA) as substrates. A deficiency results in achromotrichia which has been observed in copper deficient species other than swine.

The functions of the other isolated copper proteins have yet to be elucidated. No information with respect to changes in their relative levels with a deficiency or excess of copper has been reported.

It is evident that copper may influence many physiological processes in swine. Variation in the responses to dietary copper supplements could be attributed to differences between individual pigs, to the levels of certain constituents of the diet or to management practices associated with feeding methods or with environmental conditions.

EXPERIMENTAL

Objectives

The main objective of the experiments was to observe the effects of copper sulfate, added as 0.1% of the diet, on responses of pigs fed different diets, ad libitum or restricted, from weaning to market weight.

Four experiments were conducted; each involved different dietary conditions.

Specific experiments were designed to study:

1. a) Average daily feed intake (A.D.F.), average daily gain (A.D.G.), feed conversion efficiency (F.C.E.) and carcass measurements of pigs fed, ad libitum, eight diets containing two sources of supplementary protein, two levels of protein and for the rearing period only, two levels of supplementary copper.

   b) Blood hemoglobin levels after three weeks on trial and as individual pigs reached 45 and 88 kg. liveweight.

2. a) A.D.F., A.D.G., F.C.E. and carcass measurements of pigs fed, ad libitum, four diets containing two levels of supplementary protein and two levels of supplementary copper over the entire growing period to market weight.

   b) Blood hemoglobin levels as individual pigs attained 45 and 88 kg. liveweight.

3. a) A.D.F., A.D.G., F.C.E. and carcass measurements of male pigs fed, restricted to scale, two diets, which differed only in the level of supplementary copper, from weaning to market weight.

   b) Apparent nitrogen digestibilities after five weeks on test and as pigs reached 57 kg. liveweight.

   c) Levels of serum copper (S.C.), total serum protein (S.T.P.)

and serum protein fractions (S.P.F.) every second week during the experimental period.

d) Liver and kidney weights and copper concentrations at the termination of the experiment.

4. a) A.D.E., A.D.G., and F.C.E. of male pigs fed, to scale, diets similar to those in Experiment 3, during four sections of the growth period, weaning to 22 kg. liveweight, 23 to 45 kg., 46 to 66 kg. and 67 kg. to market weight.

b) Apparent digestibilities of energy and nitrogen during the final week of each growth section.

c) S.C., S.T.P., S.P.F. and blood hemoglobin levels at intervals during the growing period.

d) Copper concentrations in the liver, kidneys, heart, spleen, muscle and hair of pigs sacrificed at the onset of the experiment and at the end of each growth section.

e) Carcass measurements of pigs marketed at 66 and 90 kg. liveweight.

Formulation of Experimental Diets

The formulation and composition of the diets are shown in Tables 1 and 2, respectively. Mineral and vitamin supplements were included to meet or exceed the level of these nutrients recommended in the N.R.C. Nutrient Requirements of Swine (1964).

When copper was added to diets it was supplied in the form of powdered cupric sulfate pentahydrate, $CuSO_4.5H_2O$.[1] Inclusion of 908 g. for each 908 kg. of diet mixed was equivalent to a level of copper

[1] Fisher Scientific Company, Fair Lawn, New Jersey. Technical grade.

supplementation of approximately 250 parts per million (p.p.m.) of the diet.

All of the diets were mixed and bagged at the University of Alberta Livestock Farm elevator and were stored in the barn where the pigs were on experiment. Representative samples of each diet were collected in plastic bags at the time of mixing and stored for future analysis.

For digestion studies, utilizing the chromic oxide indicator method, the experimental diets involved were supplemented with 0.5% or 1.0% of powdered chromium sesquioxide, Cr_2O_3,[2] and 0.5% of corn oil to aid distribution of the indicator. Amounts of less than 18 kg. were mixed for at least 15 minutes in a Hobart Mixer,[3] while larger amounts were mixed in a Samson Mixall[4] cone mixer.

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[2]Fisher Scientific Company, Fair Lawn, New Jersey. Certified reagent grade.

[3]Hobart Manufacturing Company Ltd., Toronto, Ontario. Model D300.

[4]Chillicothe Industries Inc., Kansas City, Mo.

TABLE 1

FORMULATION OF DIETS

Experiment number	1				2		3	4
Protein source	Fishmeal		Soybean meal		Fishmeal		Fishmeal	
Protein level	High	Low	High	Low	High	Low	Low	High
Designation	HAP	LAP	HVP	LVP	HAP	LAP	LAP	HAP
Pen numbers*	1&2	3&4	5&6	7&8	B&C	B&C	B&C	B&C
Feeding method**	A.L.	A.L.	A.L.	A.L.	A.L.	A.L.	R.	R.
Ingredients, %								
Barley, ground	90.10	94.60	81.15	91.85	90.10	94.60	90.10	90.10
Soybean meal, 44%	-	-	15.00	5.00	-	-	-	-
Fishmeal, 72%	7.50	2.50	-	-	7.50	2.50	7.50	7.50
Ground limestone	0.50	0.50	0.60	0.50	0.50	0.50	0.50	0.50
Dicalcium phosphate	1.00	1.50	1.50	1.75	1.00	1.50	1.00	1.00
Salt, iodized	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Zinc sulfate	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Vitamin B mix[1]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Vitamin B_{12} (19.8 mg. kg.)	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Vitamin A[2]	+	+	+	+	+	+	+	+
Vitamin D[3]	+	+	+	+	+	+	+	+
Lyamine (20% lysine)	-	-	0.85	-	-	-	-	-

*The last designated pen in each group received the assigned diet with 0.1% copper sulfate included.

**Ad libitum -- A.L., Restricted to scale -- R.

[1]Contained the following B vitamins/kg. of vitamin mix: riboflavin, 4.4 g.; calcium pantothenate, 8.8 g.; niacin, 19.8 g.; chlorine choride, 22.0 g.; folic acid, 132.0 mg.

[2]To supply 220,000 I.U. of vitamin A/100 kg. of diet.

[3]To supply 66,000 I.U. of vitamin D_2/100 kg of diet.

TABLE 2

COMPOSITION OF DIETS

Experiment number	1				2		3	4
Protein source	Fishmeal		Soybean meal		Fishmeal		Fishmeal	
Designation	HAP	LAP	HVP	LVP	HAP	LAP	LAP	HAP
Composition (as fed) Crude protein %,(Avg.)								
Rearing	16.9	13.7	17.4	14.3	17.9	12.9	14.0	17.7
Finishing[1]	13.5	13.5	13.5	13.5	15.0	11.8	14.0	17.7
Gross energy kcal/gm.	N.O.[4]	N.O.	N.O.	N.O.	N.O.	N.O.	N.O.	3.86
D.E., kcal/gm	3.256*	3.256*	3.212*	3.212*	3.256*	3.256*	3.256*	3.256*
Calcium, %	0.76*	0.76*	0.75*	0.77*	0.76*	0.76*	0.76*	0.68
Copper, p.p.m.	N.O.	N.O.	N.O.	N.O.	N.O.	N.O.	5.4 (288)[2]	6.1 (286)[2]
Lysine, %	0.91*	0.56*	0.91*	0.51*	0.91*	0.56*	N.O.	N.O.

*Calculated value.

[1]In Experiment 1 (trial 2), Experiment 3 and Experiment 4, pigs were maintained on rearing diets except that lysine supplementation was withdrawn from groups HVP in Experiment 1. In Experiment 1 (trial 1), the four pens on the fishmeal diet received the LAP diet without copper, the four pens on the soybean meal diet received the LVP diet without copper during the finishing period.

In Experiment 2 a low protein barley was used for the finishing rations.

[2]Values for copper supplemented ration.

[3]Formulation of diets in experiments 3 and 4 were identical. Use of a low protein barley for diets of experiment 3 reduced the crude protein level in these diets.

[4]N.O. -- Not obtained.

Methods and Procedures

A) General

Unless otherwise noted all experimental pigs, from birth until allocation to an experiment were subjected to the standard management practices followed in the University of Alberta swine herd. These practices include identification by ear notching and removal of "black" teeth at birth. For prevention of anemia a 2 ml. intramuscular injection of Pigdex 100[1] (100 mg. of injectable iron/ml.) was administered at four days of age. At this time pigs received an oral supplement of approximately 0.5 g. Tylan[2] mixed in about 2 ml. of cod liver oil. Male pigs were castrated at two weeks of age and all pigs weaned at three weeks onto a prestarter ration containing approximately 24% crude protein. Between five and seven weeks of age pigs were routinely treated for ascarids, using a piperazine derivative,[3] and for skin parasites, using a lindane spray. Vaccination against erysipelas using a subcutaneous injection of a commercial vaccine[4] was carried out when the pigs were approximately nine weeks old.

All pigs in the experiments were housed in groups of four in concrete floored pens. It was not possible to conduct all the experiments in the same building but physical differences in pen size were not considered of importance as adequate floor and feeding space were provided. Each pen represented a treatment unit as pigs within a pen received the same diet.

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[1]Cyanamid of Canada Ltd., Montreal, P.Q.

[2]Elanco Products Co., Indianapolis, Ind. 22 gm tylosin/kg.

[3]Western Brand Products Ltd., Edmonton, Alberta, 616 gm/100 kg. feed.

[4]Chas. Pfizer and Company, Inc., New York, N.Y. 5 ml. dose.

In the allocation of pigs to treatment groups, uniform distribution with respect to age, sex, litter origin and initial weight, was made in so far as this was possible.

B) Experiment 1.

Two separate but concurrent trials were conducted during the period October, 1963 to February, 1964.

The first trial consisted of 32 crossbred pigs from the Hampshire, Lacombe, Landrace, Poland China and Yorkshire breeds. Four 5 to 6-week old pigs were allocated at an average weight of 11.3 kg. to each of eight dietary treatments. Each treatment group consisted of two female and two castrated male pigs.

The diets outlined in Table 1, with 0 or 0.1% copper sulfate were placed in self-feeders in each pen. Automatic watering bowls allowed free access to water at all times.

When the pigs in each pen reached an average weight of 50 kg. a dietary change was initiated. Those pigs formerly fed a fishmeal based diet received the low protein fishmeal diet (L.A.P.) with no supplementary copper. The remaining four pens were supplied the L.V.P. diet without added copper.

The second trial, which started two weeks after the first trial, was identical except that the rearing diets were fed unchanged, apart from removal of the supplementary lysine from the H.V.P. diets during the finishing period.

Pigs in both trials were housed in 2.44 m. x 3.20 m. pens in an old barn at the University Livestock Farm. The building lacked adequate temperature and ventilation control but appeared to provide suitable conditions. Straw was provided for bedding.

Individual liveweights and pen feed consumption were recorded weekly. After the weekly weighing at which a pig attained 88.6 kg. or over, it was marketed. Carcass grades and Record of Performance (R.O.P.) measurements were obtained on the basis of the system in use at the time. (Production and Marketing Branch, C.D.A., 1960, 1965).

One pig on the unsupplemented H.V.P. diet in the first trial died of mulberry heart disease after three weeks on experiment. Data from this pig were eliminated from the calculations.

C) Experiment 2.

This experiment was conducted during the period from April, 1964 through August, 1964.

Forty-eight 8-week old pigs of mixed breeding origin were allotted at an average weight of 16.4 kg. to the two diets outlined in Table 1, supplemented with 0 or 0.1% copper sulfate. Each dietary treatment was represented by three pens, each containing two female and two castrated male pigs.

Feed and water were available to the pigs at all times as in Experiment 1. The diets were fed throughout the experimental period with no change in the level of fishmeal. After the pigs in each pen reached an average weight of 50 kg. the protein level of their diet was reduced by including a barley of lower protein content in the finishing diets. Pigs were housed in an old barn as for Experiment 1.

Individual liveweights and pen feed consumption were measured weekly. After attaining a liveweight of 88.6 kg. each pig was marketed. Carcass grades and R.O.P. measurements and score were obtained.

D) Experiment 3.

Experiment 3 was conducted over the period from February, 1965 to August, 1965.

A total of 8 six-week old castrated male pigs from three Hampshire x Yorkshire litters were assigned at an average weight of 11.8 kg. to two pens. Each pen measured approximately 1.8 m. x 4.4 m. and was equipped with four individual feeding stalls. The four pigs in one pen received the diet outlined in Table 1, their littermates received the same diet supplemented with 0.1% copper sulfate. All pigs were fed individually according to a scale based on liveweight (Table 3). The individual weight of each pig obtained weekly determined the daily amount of the diet for the following seven days. Approximately one-third of the daily allocation was fed at 7:30 a.m., one-third at 12:00 noon and the remaining portion at 4:00 p.m. Water was available except during the feeding periods when each pig was confined for appoximately one hour in its feeding stall.

For the first eleven weeks of the experiment the pigs were housed in the Muttart Barn. For the remaining period they were transferred to the L-Barn but the feeding regime was maintained. Both barns allowed reasonable temperature control.

At weekly intervals records of liveweight and feed consumption for each pig were kept. On attaining a liveweight of 88.6 kg. each animal was marketed. Carcass data and R.O.P. measurements were obtained.

TABLE 3

SCALE OF FEEDING IN EXPERIMENTS 3 AND 4

LIVEWEIGHT (kg)	FEEDING SCALE (kg/day) Expt. 3	Expt. 4
Less than 9.0		0.45
9.1 13.5	0.79	0.68
13.6 18.1	0.91	0.91
18.2 22.6	1.02	1.09
22.7 27.1	1.36	1.27
27.2 31.7	1.48	1.45
31.8 36.2	1.59	1.59
36.3 40.8	1.70	1.73
40.9 45.3	1.82	1.86
45.4 49.8	1.93	2.00
49.9 54.4	2.04	2.13
54.5 58.9	2.16	2.27
59.0 63.5	2.27	2.36
63.6 68.0	2.38	2.45
68.1 72.5	2.50	2.54
72.6 77.1	2.61	2.63
77.2 to market weight	2.72	2.72

E) Experiment 4.

This experiment was conducted from February 1966 through August, 1966.

Twenty-four 3-week old Hampshire x Yorkshire castrated male pigs were assigned on the basis of litter origin and weight to six pens in the Muttart Barn. One additional male pig from four of the six litters represented, was sacrificed to provide initial levels for some of the criteria measured. The average initial weight was 5.0 kg.

The two dietary groups, Basal (Table 1) and Copper (Basal + 0.1% copper sulfate) were each allocated to three pens, (B_1, B_2, B_3 and C_1, C_2, C_3, respectively). Each pen, 1.83 m. x 4.42 m., contained four pigs which were individually fed according to the scale in Table 3 as in Experiment 3. All pigs were weighed weekly and their individual feed consumption recorded.

The experimental period was divided into four sections based on individual pig liveweight; from initial weight to 22 kg., 23 to 45 kg., 46 to 66 kg. and 67 kg. to market weight of approximately 88 kg. In each pen as one previously assigned pig reached the end of an experimental section, it was sacrificed. The six pigs at 22 kg. and those at 45 kg. liveweight were killed on the University Farm by stunning and severing the jugular vein. The pigs in the last two sections were marketed through the normal channels.

Pigs in one litter, the 80's, developed a stiff leg condition towards the end of the first section. Three of the four pigs recovered after going off feed for a period but the fourth deteriorated and was killed. Autopsy of this pig, No. 89, suggested Septic Arthritis as the underlying cause.

Metabolism Experiments

A) Using metabolism crates for total fecal collection.

Metabolism crates as used previously by Hussar (1958), measuring 71 cm. high, 104 cm. deep and 46 cm. wide, were situated approximately 60 cm. above the pen floor. They were constructed of angle iron and galvanized sheet metal. The floor of the crate consisted of an upper perforated sheet with adjacent holes, 2.2 cm. diameter, above a collection tray which had a gradual slope from the edges to a central drain hole. Liquid excreta were collected in a container situated under the tray. Solid excreta were, to a large extent, forced through the perforated floor and were retrieved from the collection tray. Feed and water containers were attached at the front of the crate.

In Experiment 4 each of the pigs sacrificed at approximately 22, 45, 67 kg. liveweight, were placed in a metabolism crate the week prior to slaughter. A fixed daily amount of diet, fed at the usual times, was allocated for the seven day period. Total collection of feces and urine was made at the end of each of the last three 24 hour periods.

Feces were collected into plastic bags and stored in the freezer compartment of a refrigerator until the collection period was over. Urine was collected in plastic buckets which were acidified daily with 25 ml. of 50% sulfuric acid. The 3-day total weight of feces and volume of urine was noted and representative samples retained for later analyses.

B) Using chromic oxide.

Moore (1957) used chromic oxide for digestibility determinations in swine and found the method to be comparable to total collection.

1. Experiment 3. Three of the four pigs in each pen were tested during the sixth week on trial, all pigs received the Cr_2O_3 supplemented diets upon reaching 56 kg. liveweight. The allocated amount of the assigned diet which included 1.0% Cr_2O_3 and 0.5% corn oil was fed to each animal for a period of three days. Approximately 150 gm. of feces was obtained four times in the last 24 hours of each trial.

2. Experiment 4. All pigs other than those in metabolism crates were tested on reaching liveweights of 22, 45, 66 and 86 kg. The assigned diets which included 1.0%*Cr_2O_3 and 0.5% corn oil were fed for a period of six days. Fecal samples were obtained five times over the final 72 hours.

All accumlated fecal samples during the collection periods were stored in plastic bags in the freezer compartment of a refrigerator. Upon completion of sample collection the composite sample was well mixed and a portion transferred to a screw-topped jar and transferred to the laboratory.

Blood Sampling

A) For hemoglobin determination.

A prominant vein on the dorsal aspect of the pigs ear was pierced with a hypodermic needle. 0.02 ml. of blood was quantitatively transferred in a blood pipet to a 20 x 150 mm tube containing 10.0 ml. of distilled water. Residual blood was removed from the pipet by rinsing with the blood solution. The tube was stoppered and transferred to the laboratory for determination.

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*In the last trial, diets contained 0.5% Cr_2O_3 + 0.5% corn oil.

Blood hemoglobin samples were obtained in Experiment 1, 2 and 4 according to the following schedule:

a) Experiment 1. One male and one female pig in each pen were sampled after three weeks on trial, an equal number were used as they attained 45 kg. liveweight. All pigs were sampled on reaching market weight.

b) Experiment 2. All pigs were sampled on reaching liveweights of 45 kg. and 88.6 kg.

c) Experiment 4. Blood samples were taken from all pigs at an average liveweight of 6.5 kg., after five weeks on experiment and on reaching 15.8 kg. The pigs destined to be sacrificed at 22, 45, 66 and 89 kg. were sampled on the day before slaughter. All remaining pigs on experiment were sampled at intermediary liveweights of 34, 57 and 79 kg.

B) For serum analyses.

Blood was withdrawn from the anterior vena cava according to the method of Carle and Dewhirst (1942). Acid washed glass syringes and standard stainless steel needles, of a size appropriate to the size of the animal, were used. Up to 10 ml. of blood were transferred to clean tubes. The blood was allowed to stand at room temperature for 1 hour then left overnight at 4 C to allow the clot to retract. The tubes were centrifuged at 2000 rpm for fifteen minutes, then the serum was transferred to copper-free glass tubes and stored frozen until analyzed.

Serum samples were obtained from pigs in Experiments 3 and 4. All pigs in Experiment 3 were sampled at bi-weekly intervals from the beginning of the trial. In Experiment 4 all pigs were bled after

three weeks on trial and at 15.8 kg. liveweight. Further samples were obtained from pre-slaughter pigs at 22, 45, 66 and 89 kg., and from the remaining pigs at 34, 57 and 79 kg. liveweight.

Tissue Sampling

In Experiment 3, the livers and kidneys of the test animals were obtained from the Swift Canadian Packing Plant shortly after slaughter.

In Experiment 4, the tissues noted below were obtained from all experimental animals:

a) Hair -- removed with clippers from the area between the right shoulder and head. White and black hair was separated.

b) Liver -- divested of the gall bladder and extraneous fat and connective tissue.

c) Kidneys -- divested of membrane, fat and extraneous connective tissue including ureters.

d) Spleen -- divested of superficial fat and blood vessels.

e) Heart -- Auricular and ventricular tissue only.

f) Muscle -- A portion, approximately 30 g. fresh weight, of the glutaeus medius from the right dorsal area.

g) Hypophysis -- minus the infundibulum.

The hair samples were stored in sealed glass tubes at room temperature until they were analyzed. Fresh weight of all other tissues was recorded before the samples were stored in sealed containers in the freezer at -16 C.

Preparation of Samples

A) Feed and fecal samples.

Feed samples were ground in a Wiley no. 9 mill[1] to pass through a

[1]A.H. Thomas Company, Philadelphia, Pennsylvania.

2 mm mesh screen. The ground samples were stored in sealed containers at a temperature not exceeding 4 C and analyzed on an air-dry (as fed) basis.

Except in Experiment 4 where fresh feces were analyzed, the fecal samples were dried in a forced air oven for 24 hours at a temperature of 65 C. The dried samples were allowed to stand for a further 24 hours at room temperature and humidity. They were then ground and stored in a sealed container as air-dry feces.

B) Tissue samples.

a) Hair -- The samples were cleaned according to the method of Goldblum et al. (1953). Approximately 0.3 gm. of hair was placed in a 50 ml. erlenmeyer with 15 ml. of 10% Tween 80. The stoppered flasks were shaken for 30 minutes on a mechanical shaker and the liquid decanted off. Successive rinses with demineralized water were performed until no persistent bubbles were evident upon shaking. The cleaned hair was transferred to a filter paper and dried in an incubator at 37 C.

b) Liver, kidneys, spleen, heart and muscle -- Each entire tissue was homogenized in an Osterizer[1] or Blender[2] with no addition of liquid. A sample of the homogenate was transferred to an aluminum weighing dish (glass vessel in the case of liver) and weighed.

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[1]John Oster Manufacturing Co., Milwaukee, Wisconsin.

[2]Waring Products Corporation, New York, N.Y.

The tissue aliquots were lyophylized for 24 hours under a minimum pressure of 30 microns of Hg. The freeze-dried samples were weighed. The difference between fresh and dried weight was used to calculate the moisture content of the fresh tissue. Dry samples were stored in sealed plastic containers at 4° C.

Chemical Analyses

Duplicate analyses were performed on each sample except in a few cases where the sample size was too small. Where results differed by 2% or over a third analysis was performed and the average value used.

Where a sample was analyzed for copper, care was taken to avoid contamination. Glassware was cleaned by soaking in a 50% nitric acid solution then rinsed in hot water, distilled water and demineralized[1] water before being oven dried. Solutions were made up with demineralized water.

A) Nitrogen.

The nitrogen content of feed, fresh or air-dried feces and urine was determined by Kjeldahl method of analysis (AOAC, 1960). A commercial Kel-Pak[2] was used to provide the required amount of catalyst for the acid digestion. The ammonia ultimately produced was retained in a 4% boric acid solution.

Where protein level was calculated, the conversion factor of

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[1]Barnstead Still and Sterilizer Co., Boston 31, Mass. Distilled water passed through Bantam Demineralizer equipped with standard cartridge.

[2]Matheson Scientific, East Rutherford, New Jersey. Supplies Hg catalyst, K_2So_4 and $CuSo_4$.

6.25 was applied to the nitrogen value.

B) Gross energy.

Determinations were made with a Parr Oxygen Bomb Calorimeter.[1]

C) Chromic oxide.

The chromic oxide content in feed and fecal samples was measured using the method of Hill and Anderson (1958) as modified by Renner (1964).* The absorbance of the final solution was measured at 448 mu by a Beckman D.U. Spectrophotometer.[2] The value was compared to known standards.

D) Copper.

a) The copper content of feed, feces and dried tissue samples was determined according to the method of Martens and Githens (1952) as modified by Andrus (1955). The procedure involved oxidation of the organic matter by the action of a mixture of HNO_3, $HClO_4$ and H_2SO_4 , and heat. Copper in the acid digest was extracted with a solution of zinc dibenzyldithiocarbamate in carbon tetrachloride. The absorbance of the resultant yellow complex was determined at 435 mu using a Spectronic 20 spectrophotometer[3] and compared to standards carried through the procedure. Some difficulty was encountered with this method; details are outlined in Appendix A.

b) Hair copper was determined using the method of Rice and Goldstein (1961), which is based on the formation of a colored

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[1]Parr Instrument Company, Moline, Illinois. Temperature changes recorded by C. Brown Electronik Recorder manufactured by Minneapolis-Honeywell Regulator Company, Philadelphia, Pennsylvania.

[2]Beckman Instruments Incorporated, Fullerton, California.

[3]Bausch and Lomb, Rochester, New York, N.Y.

*20 ml. concentrated H_2SO_4 in final dilution.

copper complex with oxalyldihydrazide. Details are given in Appendix B.

c) Serum copper concentration was measured by an adaptation of the method of Rice (1960). Protein bound copper was released by the action of hydrochloric acid. The copper formed a colored complex with oxalyldihydrazide. The absorbance of the complex was compared to standards carried through the process (Appendix C).

E) Serum total protein.

The concentration of total protein in the serum samples was determined with an Auto-Analyzer[1] equipped with a serum protein manifold. The method is basically a modification of the Biuret reaction as adapted by Stevens (1962). The developed color was measured at 550 mu and its intensity automatically recorded for each sample on a chart graduated from 0-100% transmission. The values obtained were converted to their equivalent absorbance values by applying the formula:

$$\text{Absorbance} = \log_{10}\left(\frac{100}{\%\ \text{Transmission}}\right)$$

All samples were rerun through the process with a 'blank' reagent solution to determine the correction factors for hemolysis or turbidity. The latter absorbance measurements were subtracted from the protein-biuret values to obtain the true absorbance value which was then compared to standard samples.

F) Serum protein fractions.

Serum proteins were separated electrophoretically by the Microzone

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[1]Technicon Instrument Corporation, Chauncy, New York.

method developed by Beckman Inc. (1963). A sample size of 0.5 ml. was sufficient for analysis. The method utilized a supporting membrane of cellulose acetate. Each run allowed eight samples to be separated. When veronal buffer, pH 8.6 and ionic strength 0.075, was used and a potential difference of 250 volts applied maximum separation was achieved in approximately 20 minutes.

The proteins were fixed and dyed with the recommended Ponceau-S Fixative-Dye solution and the membrane rendered transparent with a glacial acetic acid/ethanol mixture and application of heat between 100 and 110 C. The membranes were scanned with a Beckman Model RB Analytrol[1] fitted with a Microzone Scanning attachment. For each sample a scan, automatically integrated, was obtained (Figure 1).

The peaks were identified by comparison with standard serum.[2] Separation of the globulins was not complete even when conditions were varied, consequently three regions were arbitrarily designated α-, β- and γ- globulins. Separation of the regions was achieved by drawing lines vertically from the lowest point between two adjacent regions. The relative areas of the four regions, albumin and α-, β-, γ-globulins, were used to calculate the relative percentage of each fraction. From the total protein value for a serum it was possible to calculate the absolute amounts of each fraction present.

G) Blood hemoglobin.

The method used to determine hemoglobin was that of Evelyn (1936) for the determination of oxyhemoglobin. One drop of concentrated

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[1] Spinco Division, Beckman Instruments, Inc., Palo Alto, California.

[2] Hyland Laboratories, Los Angeles, California. Special Clinical Chemistry Control Serum (Human).

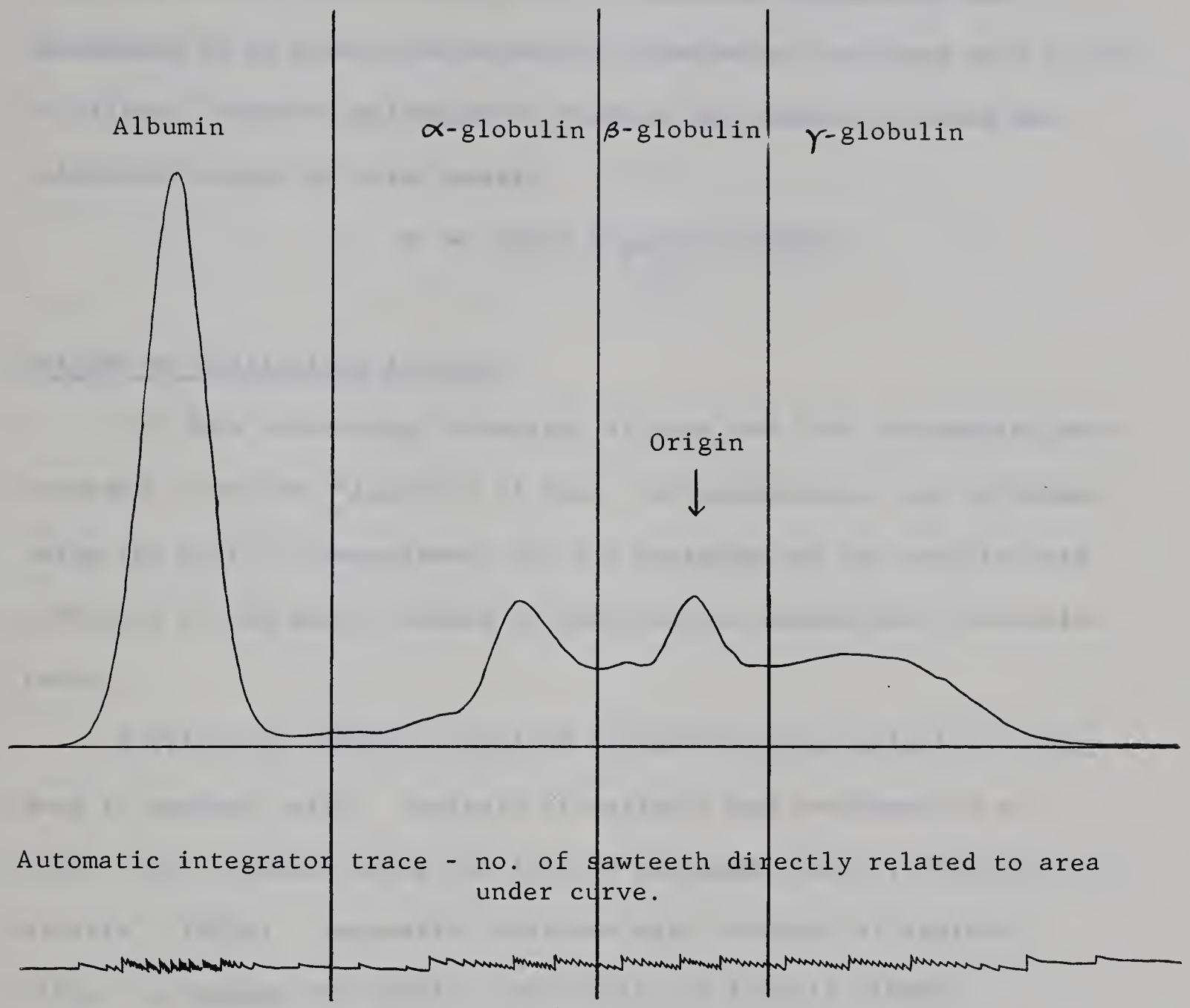
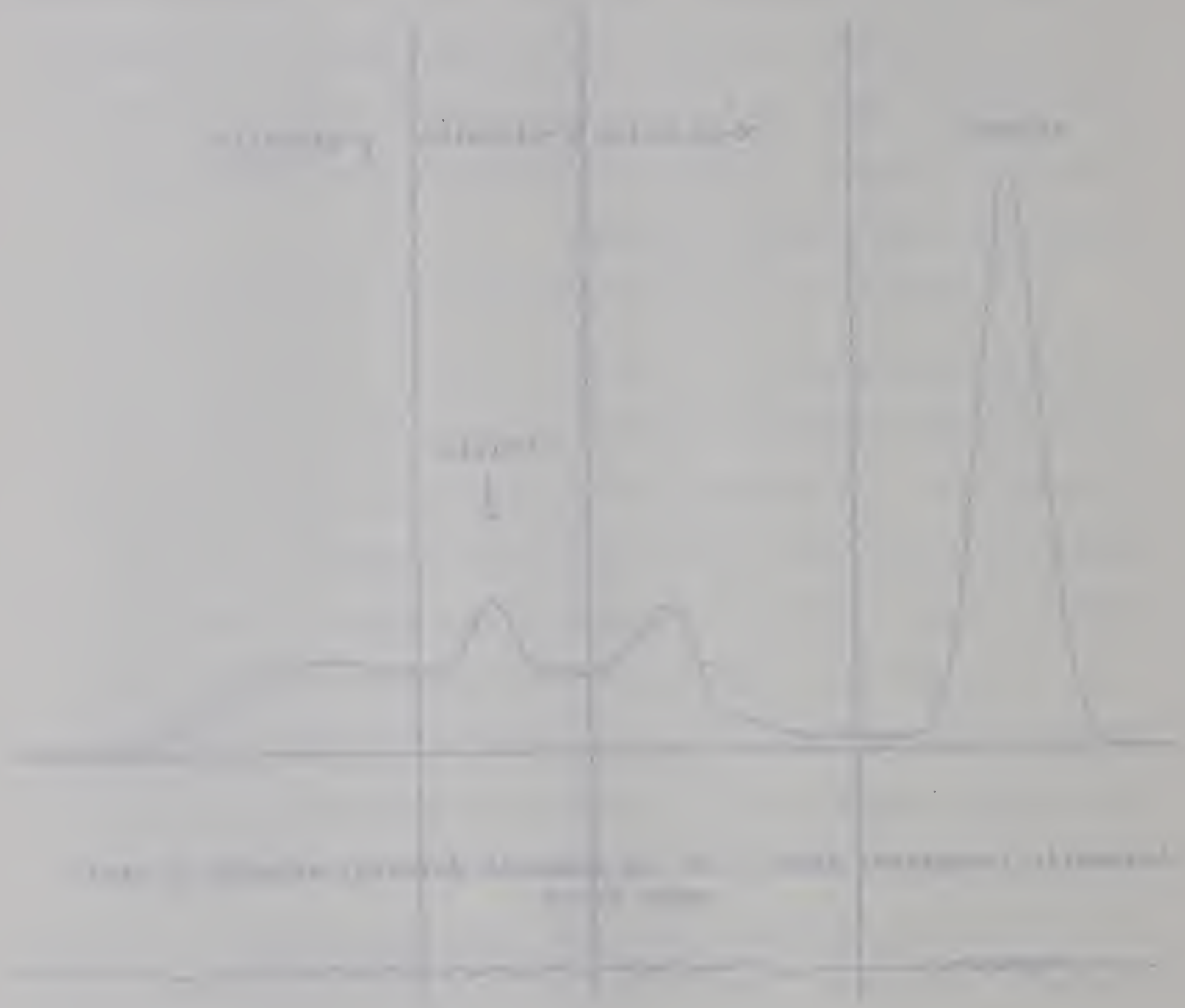


Figure 1. Typical scan of serum after Microzone electrophoresis, including the designation and separation of fractions. (Veronal buffer, pH 8.6, 0.075 ionic strength).



ammonium hydroxide was added to the solution of 0.02 ml. blood in 10 ml. distilled water. After mixing the solutions, the absorbance was determined in an Evelyn photoelectric colorimeter[1] equipped with a 540 mu filter. From the galvanometer reading the hemoglobin value was calculated using the relationship

$$X = \frac{100 \times \text{observed reading}}{2.58}$$

Methods of Statistical Analysis

All data concerning liveweight of pigs and feed consumption were recorded in pounds. Analysis of data, for convenience, was performed using the units of measurement but for presentation the results were converted to the metric system by applying the appropriate conversion factor.

Statistical analysis involved transferring the majority of the data to punched cards. Analysis of variance was performed by an I.B.M. 7040 computer using the library programme BMDO2V, University of Alberta, (1965a). Regression analyses were obtained by applying library programme REG (G2011), University of Alberta (1965b).

Missing data were replaced by the average value of animals on the same treatment. This was considered to be the best estimate of the missing value.[2] One degree of freedom was dropped for each substituted value. For sections 2 and 3, in Experiment 4, values for pig No. 89 were missing on account of its early death. Other substituted values in tables are enclosed within parentheses.

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[1] Rubicon Company, Philadelphia, Penn.

[2] Dr. R.T. Hardin, personal communication.

A composite error term was used for most of the analyses of variance. It consisted of the sum of squares of all third order interactions plus the residual sum of squares.

A probability of 0.05 or less was judged to be significant, probabilities of 0.01 or less were deemed highly significant.

Some differences between means were analyzed by applying Duncans new multiple range test (Li, 1957).

RESULTS AND DISCUSSION

Except where individual observations were regarded to be of value, averages of the data are reported. Those values which on analysis were found to be statistically significant are indicated on the tables.

Growth Data

The experimental values for average daily feed (A.D.F.), average daily gain (A.D.G.) and efficiency of feed conversion (F.C.E.) are summarized for Experiments 1, 2 and 3, and 4 in Tables 4, 6 and 8, respectively. The growth curves for the basal and copper supplemented pigs in Experiment 4 were calculated from the average weekly liveweight increment of all residual pigs (Figure 2).

The overall effects of copper supplementation as well as protein level and source on A.D.F., A.D.G. and F.C.E. are presented for Experiment 1 in Table 5, for Experiments 2 and 3 in Tables 6 and 7 and for Experiment 4 in Table 8.

In Experiment 1, copper reduced A.D.F., increased A.D.G. and improved F.C.E. during the rearing period. In the finishing period, where copper was included only in diets in Trial 2, both A.D.F. and A.D.G were reduced while F.C.E was unaffected. Over the entire experimental period, copper was beneficial only in Trial 2.

In Experiments 2 and 3 no consistent overall effects of copper were noticed. The data for Experiment 4 indicated that supplemental copper was beneficial for A.D.G. and F.C.E. significantly during the first period, up to an average liveweight of 22 kg. In the following periods the advantages of adding copper to the diet decreased and in the final and overall periods copper appeared to adversely affect the rate of gain measurements.

TABLE 4

EXPERIMENT 1. DAILY FEED CONSUMPTION, GAIN AND FEED CONVERSION EFFICIENCY MEANS.

	Experiment no.		1							
	Protein source		Barley-Fishmeal				Barley-Soybean meal			
	Protein level		High		Low		High		Low	
	Copper supplement		0	+	0	+	0	+	0	+
Trial 1	No. of pigs		4	4	4	4	3	4	4	4
	Avg. initial age	(days)	44	43	42	42	40	43	43	44
	Avg. initial wt.	kg.	11.2	11.3	11.0	11.6	10.7	11.6	11.0	11.5
Rearing	Avg. daily feed	kg.	1.71	1.58	1.74	1.54	1.84	2.01	1.70	1.69
period(Start	Avg. daily gain	kg.	0.58	0.70	0.50	0.55	0.67	0.68	0.51	0.49
to 47 kg.)	Feed/kg. gain	kg.	2.97	2.26	3.52	2.82	2.75	2.95	3.36	3.46
Finishing	Avg. daily feed	kg.	3.34	3.42	3.22	3.43	4.02	3.56	3.44	4.00
period (47	Avg. daily gain	kg.	0.86	0.88	0.86	0.83	0.96	0.88	0.92	0.89
kg.to market)	Feed/kg.gain	kg.	3.88	3.90	3.73	4.15	4.20	4.03	3.72	4.49
Overall	Avg. daily feed	kg.	2.45	2.40	2.36	2.41	2.83	2.81	2.43	2.68
period	Avg. daily gain	kg.	0.70	0.78	0.65	0.67	0.80	0.79	0.68	0.66
	Feed/kg. gain	kg.	3.47	3.08	3.64	3.57	3.54	3.58	3.57	4.06
Trial 2	No. of pigs		4	4	4	4	4	4	4	4
	Avg. initial age	(days)	45	47	46	45	45	46	48	46
	Avg. initial wt.	kg.	10.9	10.5	11.0	10.5	10.9	10.5	11.0	10.9
Rearing	Avg. daily feed	kg.	1.84	1.67	1.75	1.69	1.84	1.92	1.67	1.72
period(start	Avg. daily gain	kg.	0.67	0.69	0.51	0.55	0.62	0.70	0.47	0.57
to 50 kg.)	Feed/kg. gain	kg.	2.73	2.42	3.40	3.08	2.97	2.75	3.58	3.04
Finishing	Avg. daily feed	kg.	3.42	3.28	3.72	3.43	3.50	3.28	3.31	3.10
period (50	Avg. daily gain	kg.	0.86	0.82	0.94	0.87	0.83	0.84	0.89	0.77
kg.to market)	Feed/kg. gain	kg.	3.98	4.00	3.94	3.92	4.22	3.92	3.71	4.03
Overall	Avg. daily feed	kg.	2.58	2.43	2.46	2.33	2.58	2.57	2.25	2.31
period	Avg. daily gain	kg.	0.76	0.75	0.67	0.67	0.71	0.76	0.62	0.65
	Feed/kg. gain	kg.	3.39	3.24	3.67	3.49	3.62	3.36	3.65	3.54

TABLE 5

EXPERIMENT 1. GROWTH DATA MEANS WITH RESPECT TO DIETARY VARIABLES.

		Protein source		Protein level		Copper level	
		Fishmeal	Soybean	High	Low	0	+
Average daily feed, kg.							
Rearing period	Trial 1	1.64	1.81	1.78	1.67	1.75	1.70
	Trial 2	1.74	1.79	1.82	1.71	1.77	1.75
Finishing period	Trial 1	3.35	3.75	3.58	3.52	3.51	3.60
	Trial 2	3.46	3.30	3.37	3.39	3.49	3.27
Overall period	Trial 1	2.40	2.69	2.62	2.47	2.52	2.57
	Trial 2	2.45	2.43	2.54	2.34	2.47	2.41
Average daily gain*, kg.							
Rearing period	Trial 1	0.58	0.59	0.66	0.51	0.56	0.60
	Trial 2	0.60	0.59	0.67	0.52	0.57	0.63
Finishing period	Trial 1	0.86	0.91	0.89	0.87	0.90	0.87
	Trial 2	0.87	0.83	0.84	0.87	0.88	0.82
Overall period	Trial 1	0.70	0.73	0.77	0.66	0.71	0.72
	Trial 2	0.71	0.68	0.74	0.65	0.69	0.71
Feed conversion feed/kg. gain, kg.							
Rearing period	Trial 1	2.89	3.13	2.73	3.29	3.15	2.87
	Trial 2	2.91	3.08	2.72	3.27	3.17	2.82
Finishing period	Trial 1	3.91	4.11	4.00	4.02	3.88	4.14
	Trial 2	3.96	3.97	4.03	3.90	3.96	3.97
Overall period	Trial 1	3.44	3.69	3.42	3.71	3.55	3.57
	Trial 2	3.45	3.54	3.40	3.59	3.58	3.41

*In Trial 1 -- The effects of sex, period x sex and source x sex were significant ($P < 0.05$), the period, protein level and period x protein level had a highly significant effect ($P \leq 0.01$) upon the average daily gain of individual pigs.

In Trial 2 -- The effect of protein level was significant; period, period x protein level and period x copper level were highly significant.

In both trials the effect of copper level on average daily gain of pigs was not significant.

TABLE 6

EXPERIMENTS 2 AND 3. DAILY FEED CONSUMPTION, GAIN AND FEED CONVERSION EFFICIENCY MEANS.

	Experiment no.		2				3	
	Protein source		Barley-Fishmeal				Barley-Fishmeal	
	Protein level		High		Low		Low	
	Copper supplement		0	+	0	+	0	+
	No. of pigs		12	12	12	12	4	4
	Avg.initial age (days)		60	60	60	60	43	43
	Avg. initial wt.	kg.	16.3	16.2	16.4	16.2	11.4	12.3
Rearing period (Start to 48 kg.)	Avg. daily feed	kg.	1.87	1.90	1.83	1.84	1.24	1.28
	Avg. daily gain	kg.	0.70	0.76	0.59	0.57	0.42	0.43
	Feed/kg. gain	kg.	2.66	2.51	3.07	3.20	2.97	2.96
Finishing period (48 kg. to market)	Avg. daily feed	kg.	3.22	3.29	3.28	3.36	2.40	2.38
	Avg. daily gain	kg.	0.86	0.88	0.86	0.88	0.64	0.66
	Feed/kg. gain	kg.	3.73	3.74	3.80	3.82	3.73	3.63
Overall period	Avg. daily feed	kg.	2.58	2.65	2.52	2.55	1.74	1.75
	Avg. daily gain	kg.	0.79	0.82	0.72	0.72	0.51	0.53
	Feed/kg. gain	kg.	3.28	3.22	3.49	3.56	3.38	3.31

TABLE 7

EXPERIMENT 2. GROWTH DATA MEANS WITH RESPECT TO DIETARY VARIABLES.

	Protein Level		Copper Level	
	High	Low	0	+
Average daily feed, kg.				
Rearing period	1.88	1.83	1.85	1.87
Finishing period	3.25	3.32	3.25	3.32
Overall period	2.61	2.53	2.55	2.60
Average daily gain, kg.				
Rearing period	0.73	0.58	0.64	0.66
Finishing period	0.87	0.87	0.86	0.88
Overall period	0.80	0.72	0.75	0.77
Feed conversion feed/kg. gain, kg.				
Rearing period	2.58	3.13	2.86	2.85
Finishing period	3.73	3.81	3.77	3.78
Overall period	3.25	3.52	3.38	3.39

TABLE 8

EXPERIMENT 4. DAILY FEED CONSUMPTION, GAIN AND FEED CONVERSION EFFICIENCY MEANS.

Experiment no.		4	
Protein source		Barley-Fishmeal	
Protein level		High	
Copper supplement		0	+
Initial no. of pigs		12	12
Avg. initial age (days)		27	27
Avg. initial wt. (kg.)		5.6	5.5
First period (Start to 22 kg.)	No. of pigs	12	12
	Avg. daily feed kg.	0.61	0.64
	Avg. daily gain kg.	0.27	0.30*
	Feed/kg. gain kg.	2.25	2.14*
Second period (23 - 45 kg.)	No. of pigs	9	8
	Avg. daily feed kg.	1.48	1.46
	Avg. daily gain kg.	0.52	0.54
	Feed/kg. gain kg.	2.87	2.71
Third period (46 - 66 kg.)	No. of pigs	6	5
	Avg. daily feed kg.	2.13	2.12
	Avg. daily gain kg.	0.63	0.60
	Feed/kg. gain kg.	3.52	3.49
Fourth period (67 kg. to market)	No. of pigs	3	3
	Avg. daily feed kg.	2.57	2.61
	Avg. daily gain kg.	0.63	0.59
	Feed/kg. gain kg.	4.08	4.47
Overall period	No. of pigs	3	3
	Avg. daily feed kg.	1.57	1.61
	Avg. daily gain kg.	0.48	0.49
	Feed/kg. gain kg.	3.27	3.31

*Significant at $P < 0.05$.

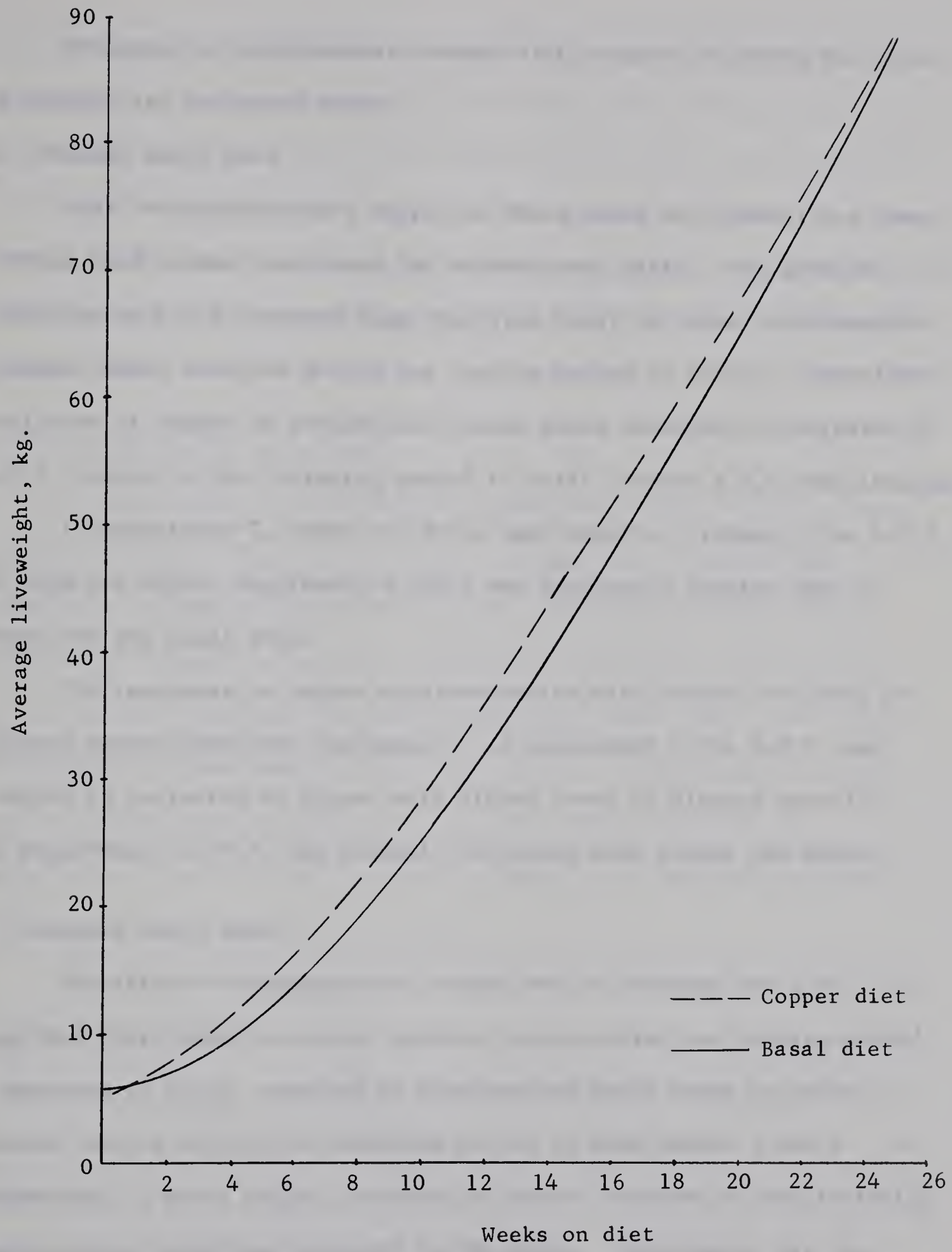
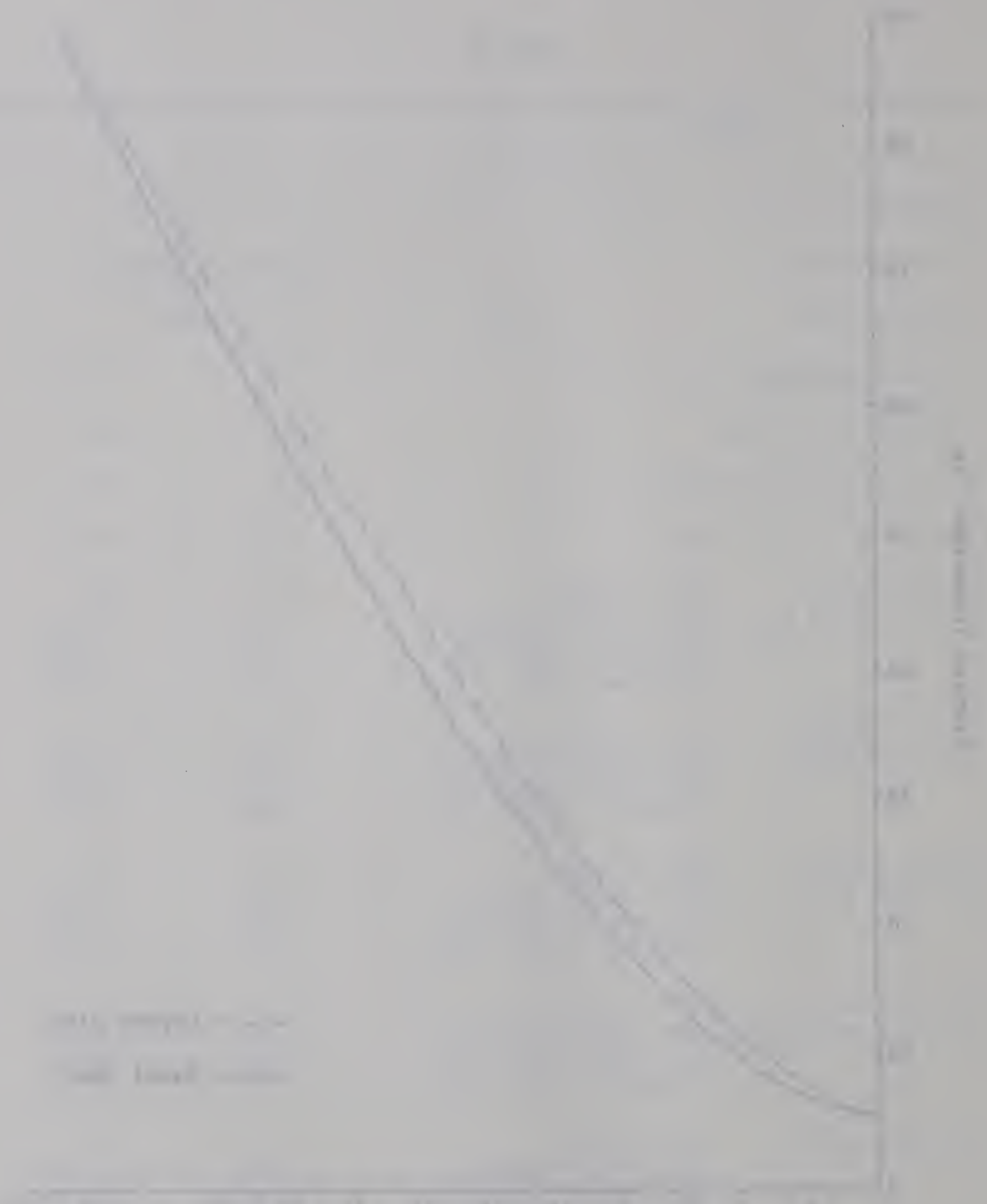


Figure 2. Experiment 4 -- Accumulated average liveweight gains of basal and copper fed pigs.



Responses to supplementary copper with respect to source and level of protein are indicated below:

A) Average daily feed

Pigs fed supplementary copper in diets based on fishmeal had lower average feed intake than those fed soybean meal diets. The greatest reduction in A.D.F. between pigs receiving basal or copper supplemental fishmeal diets occurred during the rearing period in Trial 1, Experiment 1. Inclusion of copper in soybean meal based diets resulted in increases in A.D.F. except in the finishing period in Trial 2 where A.D.F. was lowered.

In Experiment 2, where all diets were based on fishmeal, the A.D.F. of pigs fed copper supplemented diets was marginally greater than of those fed the basal diets.

The responses to copper supplementation with respect to level of dietary protein were not consistent. In Experiment 1 the A.D.F. was reduced by inclusion of copper with either level of dietary protein. In Experiment 2,A.D.F. was slightly increased when copper was added.

B) Average daily gain

The effect of supplementary copper was to increase the A.D.G. for pigs fed diets based on either protein source during the rearing period. A decrease in A.D.G. occurred in supplemented diets based on either protein source during the finishing period in Experiments 1 and 4. In Experiments 2 and 3 slight increases in A.D.G. occurred in the finishing period where copper was included in the diets. Improvement for the overall period was observed for all pigs on fishmeal based diets except those in Trial 2, Experiment 1. For the soybean meal fed groups a decrease in A.D.G. was observed in Trial 1 and an increase in A.D.G. in Trial 2 for the overall period when copper was included in the diets.

During the rearing period in Experiment 1 pigs fed either level of dietary protein had an increased A.D.G. when copper was included. In the finishing period copper had a slight adverse effect which was more pronounced in pigs fed the lower level of protein. However, for the overall period copper-fed pigs had a slightly higher average rate of gain. In Experiment 2 copper supplementation was beneficial only for the pigs fed the higher level of protein.

C) Feed conversion efficiency

In Experiment 1 the beneficial effects of copper on F.C.E. were similar to those on A.D.F. and A.D.G. Copper was of benefit when included in fishmeal based diets during both trials for the rearing and overall periods. Pigs fed soybean meal based diets had improved F.C.E. in the rearing and overall periods only when copper was added throughout the experimental period (Trial 2). In Experiment 2 copper had no overall effect upon F.C.E. For the overall periods in Experiments 3 and 4 the F.C.E. was increased and decreased, respectively, when copper was included in the diets.

Pigs fed either high level of dietary protein benefited from inclusion of copper during all periods in Experiment 1. Pigs on the lower protein diets converted feed more efficiently during the rearing period when copper was included and in the overall period only in Trial 2. There was minimal benefit in Experiment 2 for pigs fed the higher protein diets supplemented with copper.

The results obtained from Experiments 1 and 2 where copper was included in ad libitum fed diets agree for the most part with previous reports in the literature. The effects of supplementary copper were most pronounced during the rearing period as has previously been reported

(Barber et al., 1955; Wallace et al., 1962). Data from Trial 1, Experiment 1, indicated that the improvements during the rearing period in A.D.G. and F.C.E. were often of sufficient magnitude to be reflected in the gain measurements over the entire experimental period.

In general, supplementary copper was of greater benefit for pigs receiving fishmeal rather than soybean meal diets and with relatively high protein diets than with lower protein diets. Lucas, Livingstone and Boyne (1962) obtained similar results with pigs fed to scale.

It has been assumed that part of the effect of supplementary copper on growth measurements is a result of increasing the appetite (Sharpe and Dent, 1966). In the two experiments where feed was available ad libitum inclusion of copper resulted in a decrease in feed intake of fishmeal based diets and an increase in feed intake in soybean meal based diets in Experiment 1. In Experiment 2 feed intake was slightly increased.

The results obtained in Experiments 1 and 2 suggested that where copper is included at a fixed level over the entire growing period, for maximum benefits the dietary protein levels should not be reduced during the finishing period.

Experiments 3 and 4 involved fishmeal based diets fed restricted to scale. The scales of feeding were probably over-restrictive as the figures for average daily gain indicate when the data are compared to results in Experiment 2. The responses to supplementary copper in Experiment 3 were lower than in Experiment 4. The higher levels of protein in the diets in Experiment 4 could have accounted for the differences in responses. The data in Table 8 and the growth curves for Experiment 4 (Figure 2) tend to confirm that copper supplementation is of maximum benefit in the early stages of growth.

Carcass data

The mean values for carcass measurements from market pigs in all experiments are recorded in Table 9.

Supplementary copper reduced average age to market for ad libitum fed pigs but had an inconsistent effect on marketing age when pigs were fed to scale.

The dressing percentages in all but Experiment 4 were reduced in pigs fed copper supplemented diets irrespective of the dietary protein source or level. These results are in contrast to those of other workers (Lucas and Calder, 1957a; Barber, Braude and Mitchell, 1960; Allen et al., 1961) who reported increases in dressing percentages. However, data from Experiment 4, which was similar in design to the published experiments, gave similar results, i.e. increased dressing percentage from copper supplements.

There was a general tendency for pigs which received supplemental copper to have shorter carcass lengths, an effect of copper previously observed by Barber, Braude and Mitchell (1960) and Allen et al. (1961). Reduction in average backfat thickness occurred in all supplemented groups except those receiving the high-protein soybean meal diets. This is in contrast to reports that copper supplementation of diets increased backfat thickness (Barber et al., 1957; Barber, Braude and Mitchell, 1960; Allen et al., 1961).

Loin eye area was increased in pigs fed ad libitum copper supplemented diets containing the higher level of protein. When the diets were fed to scale loin eye area was increased in all supplemented groups. A similar effect upon the eye muscle was reported by Braude et al., (1962) and Lucas et al., (1962).

The effect of supplementary copper upon carcass grades and R.O.P.

TABLE 9

EXPERIMENTS 1, 2, 3 and 4. CARCASS DATA.

Experiment no.		1				2							
Protein source		Barley-Fishmeal				Barley-Soybean Meal				Barley-Fishmeal			
Protein level		High		Low		High		Low		High		Low	
Copper supplement		0	+	0	+	0	+	0	+	0	+	0	+
No. of pigs		8	8	8	8	7	8	8	8	12	12	12	12
Carcass data													
Avg. age to market (days)		154	150	165	163	150	148	168	166	154	150	163	164
Avg. final wt.	kg.	91.7	90.3	90.0	91.0	92.3	91.7	92.1	90.9	90.6	91.5	90.1	91.9
Avg. hot carcass wt.	kg.	71.0	68.2	69.8	69.2	70.8	70.7	71.2	69.1	69.6	68.8	69.4	70.3
Dressing percentage	%	77.5	75.5	77.6	76.0	76.7	77.2	77.3	76.0	76.8	75.2	77.0	76.5
Carcass length	cm.	77.5	76.7	75.4	76.2	78.0	75.9	77.2	77.2	75.7	75.2	75.4	75.2
Avg. backfat	cm.[2]	3.66	3.12	3.81	3.56	3.56	3.66	3.96	3.58	3.45	3.25	3.66	3.58
Area of loin	sq. cm.	22.1	25.4	21.7	21.2	22.4	25.1	21.2	21.9	26.5	27.7	24.5	23.2
Carcass grades													
A		2	1	0	1	3	2	3	3	5	2	2	2
B		6	7	6	7	2	4	2	4	7	10	10	10
C		0	0	2	0	2	2	3	1	-	-	-	-
Total R.O.P. score	%[3]	53	81	47	60	55	55	46	56	66	74	52	56
No. of carcasses with soft fat		0	7	1	7	0	1	0	3	0	10	1	8

[1]Pigs marketed at 68 kg. liveweight.

[2]Average of measurements at shoulder, back and loin.

[3]Carcasses from Experiments 1 and 2 by R.O.P. (1960), from Experiments 3 and 4 by R.O.P. (1965).

*$P < 0.05$.

scores present conflicting results. In general, the inclusion of copper in diets improved the R.O.P. score to a greater extent than was reflected in the grades obtained. Part of the discrepancy was the result of normal skin pigmentation in pigs from Hampshire crosses, which prevented affected carcasses obtaining an A grade. Observed softness in the consistency of the carcass fat resulted in downgrading of many of the copper supplemented pigs fed ad libitum. Taylor and Thomke (1964) reported that inclusion of 250 p.p.m. copper in ad libitum fed diets significantly increased the Iodine Number and degree of softness of the depot fat in pigs. Similar results were obtained in the present Experiments (1 and 2) with the greatest effect observed in the copper supplemented fishmeal rations (Table 9). This aspect of copper metabolism is currently being investigated in the Department of Animal Science at the University of Alberta.

Metabolism trials

The results of the metabolism trials conducted in Experiments 3 and 4 are summarized in Tables 10, 11 and 12. Nitrogen analyses were performed on both fresh (a), and air dried (b) fecal samples and apparent digestibilities, (a) and (b) respectively, after converting to a common basis were calculated. In many cases the apparent digestibility was higher when data from dried fecal samples were used. It was considered that an obvious error could arise from the loss of nitrogen during the drying period. Any such loss would tend to be erroneously regarded as absorbed nitrogen and would lead to an over estimate of nitrogen digestibility. Contamination of fecal samples with urine was more liable to occur where metabolism crates were used. The greater deviation between the (a) and (b) apparent digestibilties

TABLE 10

EXPERIMENT 4. APPARENT ENERGY AND NITROGEN DIGESTIBILITIES AND NITROGEN AND COPPER RETENTION MEANS BY TOTAL COLLECTION METHOD.

Trial no.			1		2		3	
Diet			Basal	Copper	Basal	Copper	Basal	Copper
Avg. daily feed		kg.	1.27	1.27	1.84	1.80	2.38	2.48
No. of pigs			3	3	3	3	3	2
Avg. final weight		kg.	25.2	26.0	47.3	47.4	69.0	68.1
Apparent digestibility[1]								
Energy	(b)*	%	79.5	80.5	81.1	80.0	84.7	81.4
Nitrogen	(a)*	%	74.3	77.1	77.3	77.8	85.9	82.3
	(b)*	%	78.5	80.1	82.3	81.7	87.0	83.9
Apparent retention								
Nitrogen[2]	(a)*	g./day	17.0	16.7	20.8	21.2	23.9	16.6
	(b)*	g./day	18.4	17.4	23.4	23.3	24.6	17.8
Copper[3]	(b)*	mg./day	1.5	102.0	3.4	96.0	5.9	137.0

[1]Apparent percentage of feed component absorbed from digestive tract.
e.g. For energy $\frac{\text{(Energy consumed - Fecal energy) x 100}}{\text{Energy consumed}}$

[2]Total amount of feed nitrogen less amount of nitrogen excreted in feces and urine.

[3]Copper intake less fecal copper.

*a -- data derived from analysis of fresh feces.
*b -- data derived from analysis of dried feces.

TABLE 11

EXPERIMENT 3. APPARENT NITROGEN DIGESTIBILITIES USING THE CHROMIC OXIDE INDICATOR METHOD.

Trial no.		1		2	
Diet		Basal	Copper	Basal	Copper
Avg. daily feed	kg.	1.06	1.10	2.16	2.16
No. of pigs		3	3	4	4
Avg. final weight	kg.	23.2	24.9	60.1	61.4
Apparent digestibility					
Nitrogen (b)	%	76.3	73.6	72.6	72.3

TABLE 12

EXPERIMENT 4. APPARENT NITROGEN DIGESTIBILITIES USING THE CHROMIC OXIDE INDICATOR METHOD.

Trial no.		1		2		3		4	
Diet		Basal	Copper	Basal	Copper	Basal	Copper	Basal	Copper
Avg. daily feed	kg.	1.27	1.27	1.86	1.86	2.54	2.54	2.72	2.72
No. of pigs		9	8	6	5	3	3	3	3
Avg. final weight	kg.	24.8	25.1	46.7	45.7	69.6	67.6	86.7	86.7
Apparent digestibility									
Nitrogen (a)	%	70.6	75.5	72.4	71.8	67.8	69.1	71.3	72.1
(b)	%	72.5	75.2	73.5	72.6	70.1	69.1	73.7	72.5

of nitrogen for pigs in the metabolism crates tended to emphasize the chance of error if dried feces were employed for nitrogen analysis.

A) Using metabolism crates.

The mean values are indicated in Table 10. In the first trial with 25 kg pigs copper supplementation resulted in increases in apparent digestibilities of energy and nitrogen, from 79.5 to 80.5% and 74.3 to 77.1%,respectively. The apparent retention of copper increased for pigs receiving the copper supplemented diet.

When 47 kg pigs were tested a lower apparent digestibility of energy (81.2 to 80.0%) and slightly higher (77.3-77.8%) apparent nitrogen digestibility was observed. Nitrogen retention was slightly higher and copper retention markedly higher with copper supplemented diets.

The trends observed in the first two trials were emphasized in pigs tested in the third trial at 68 kg. liveweight. Lower apparent digestibilities for energy and nitrogen and less apparent nitrogen retention occurred in pigs fed the copper supplemented diets. However, apparent retention of copper was increased.

The data obtained in these trials suggest that the benefit obtained from supplementing rearing rations with copper might result from the action of copper in increasing digestibilities of the energy and protein components of the diet. The data concerning apparent retention of copper must be considered as indicative of differences rather than an absolute measure.

B) Using chromium sesquioxide.

Data from the metabolism trials utilizing the chromic oxide indicator method, are summarized in Tables 11 and 12, respectively.

Experiments 3 and 4 differed principally in the level of dietary protein, 14% in Experiment 3 and 17.7% in Experiment 4. The trials in Experiment 4 gave lower values for apparent nitrogen digestibility, but similar trends to the observations obtained from pigs of the same liveweight in the metabolism crates. The results from pigs in Experiment 3 are in contrast to those from Experiment 4 and suggest that copper supplementation had an adverse effect upon apparent digestibility of nitrogen, particularly in the early stages of growth. Digestibility values in Experiment 3 were slightly higher than those obtained at comparative periods in Experiment 4.

Reports in the literature (Kirchgessner and Giessler, 1961; Braude, 1965) have indicated a significant increase in daily nitrogen retention and apparent nitrogen digestibility when 250 p.p.m. copper was included in the diets. Braude (1965) reported that the beneficial effects of copper on apparent digestibility and nitrogen retention were observed only in pigs which showed a marked growth response associated with copper supplementation.

Blood analyses

While performing analyses on the serum samples from Experiment 3 it was found that some samples had become slightly dehydrated. The effect of freezing had loosened some of the stoppers of the sample tubes and consequently loss of moisture had occurred over the storage period. The main effect resulted in concentration of the serum proteins yielding erroneously high values for serum total protein and protein fractions. Consequently the data from Experiment 3 were omitted. Serum copper values in Experiment 3 were presumably affected, but have been included in this report as the observed changes were almost identical to those

obtained in Experiment 4.

A) Hemoglobin values.

The results of hemoglobin analyses are presented in Tables 13, 14 and 15. Supplemental dietary copper had no consistent effect upon the hemoglobin values at the time intervals measured in Experiments 1, 2 and 4.

Miller et al. (1961b) have reported measurements of hemoglobin levels in normal pigs. The experimental values obtained were comparable to those reported in the forementioned reference. Supplemental copper has been reported to result in lowered hemoglobin values (Bunch et al., 1961; Ritchie et al., 1963); no such effect was apparent in the conducted experiments.

B) Serum copper levels.

The average values obtained for serum copper of the basal and the copper supplemented pigs in Experiment 3 are represented in Figure 3; The average values in Experiment 4 are represented in Figure 4.

At the beginning of the trial all pigs in Experiment 4 had serum copper levels of approximately 210 ug./100 ml. serum. After the third week the average serum copper level of the basal fed pigs was reduced to approximately 170 ug./100 ml. while the average copper level of the supplemented pigs increased to approximately 225 ug./100 ml. serum. The level in the basal fed pigs returned to the initial value as pigs reached 25 kg. liveweight and thereafter showed a continuous slight increase over the growing period to a final value of approximately 250 ug./100 ml. The serum copper levels in the supplemented pigs increased over the growing period to a maximum average value of approximately 300 ug./100 ml. at about 70 kg. liveweight. Thereafter, copper

TABLE 13

EXPERIMENT 1. MEAN VALUES FOR HEMOGLOBIN AND THE EFFECTS OF DIETARY VARIABLES.

Experiment no.	1							
Protein source	Barley-Fishmeal				Barley-Soybean meal			
Protein level	High		Low		High		Low	
Copper supplement	0	+	0	+	0	+	0	+
Trial 1 After 3 wks.								
No. of pigs	2	2	2	2	2	2	2	2
Hemoglobin g./100 ml.	10.58	12.17	12.26	10.77	11.04	11.80	12.08	9.29
At 46 kg.								
No. of pigs	2	2	2	2	2	2	2	2
Hemoglobin g./100 ml.	13.15	12.72	12.03	12.32	13.66	13.35	11.59	13.04
At marketing								
No. of pigs	4	4	4	4	3	4	4	4
Hemoglobin g/100 ml.	12.00	12.90	12.97	13.14	12.13	11.79	14.46	11.30
Trial 2 After 3 wks.								
No. of pigs	2	2	2	2	2	2	2	2
Hemoglobin g./100 ml.	10.45	11.79	9.76	10.26	10.93	12.19	10.09	11.06
At 46 kg.								
No. of pigs	2	2	2	2	2	2	2	2
Hemoglobin g./100 ml.	14.19	14.51	12.96	11.98	13.21	13.93	12.24	11.76
At marketing								
No. of pigs	4	4	4	4	4	4	4	4
Hemoglobin g./100 ml.	13.36	12.77	11.48	12.62	12.49	12.65	12.73	13.44

Marginal means		Trial 1			Trial 2		
		At 3 wks.	At 46 kg.	At market	At 3 wks.	At 46 kg.	At market
Protein level	Low	11.10	12.25	12.97	10.29	12.24	12.57
	High	11.40	13.22	12.18	11.34	13.96*	12.82
Copper supplement	0	11.49	12.61	12.86	10.31	13.15	12.52
	+	11.01	12.86	12.28	11.33	13.05	12.87
Sex	Male	11.29	12.55	12.49	10.92	13.09	13.20*
	Female	11.21	12.92	12.66	10.72	13.11	12.19
Protein source	Fishmeal	11.45	12.56	12.75	10.56	13.41	12.56
	Soybean	11.06	12.91	12.39	11.07	12.79	12.83

*$P < 0.05$.

TABLE 14

EXPERIMENT 2. MEAN VALUES FOR HEMOGLOBIN AND THE EFFECTS OF DIETARY VARIABLES.

Experiment no.	2			
Protein source	Barley-Fishmeal			
Protein level	High		Low	
Copper supplement	0	+	0	+
Sampling time				
1) At 46 kg.				
No. of pigs	12	12	12	12
Hemoglobin g./100 ml.	12.99	12.31	12.03	12.16
2) At marketing				
No. of pigs	12	12	12	12
Hemoglobin g./100 ml.	12.97	13.46	12.51	12.74

Marginal means

Protein level	Low	12.36
	High	12.93*
Copper supplement	0	12.62
	+	12.67
Sex	Male	12.48
	Female	12.82
Sampling time	At 46 kg.	12.38
	At market	12.92*

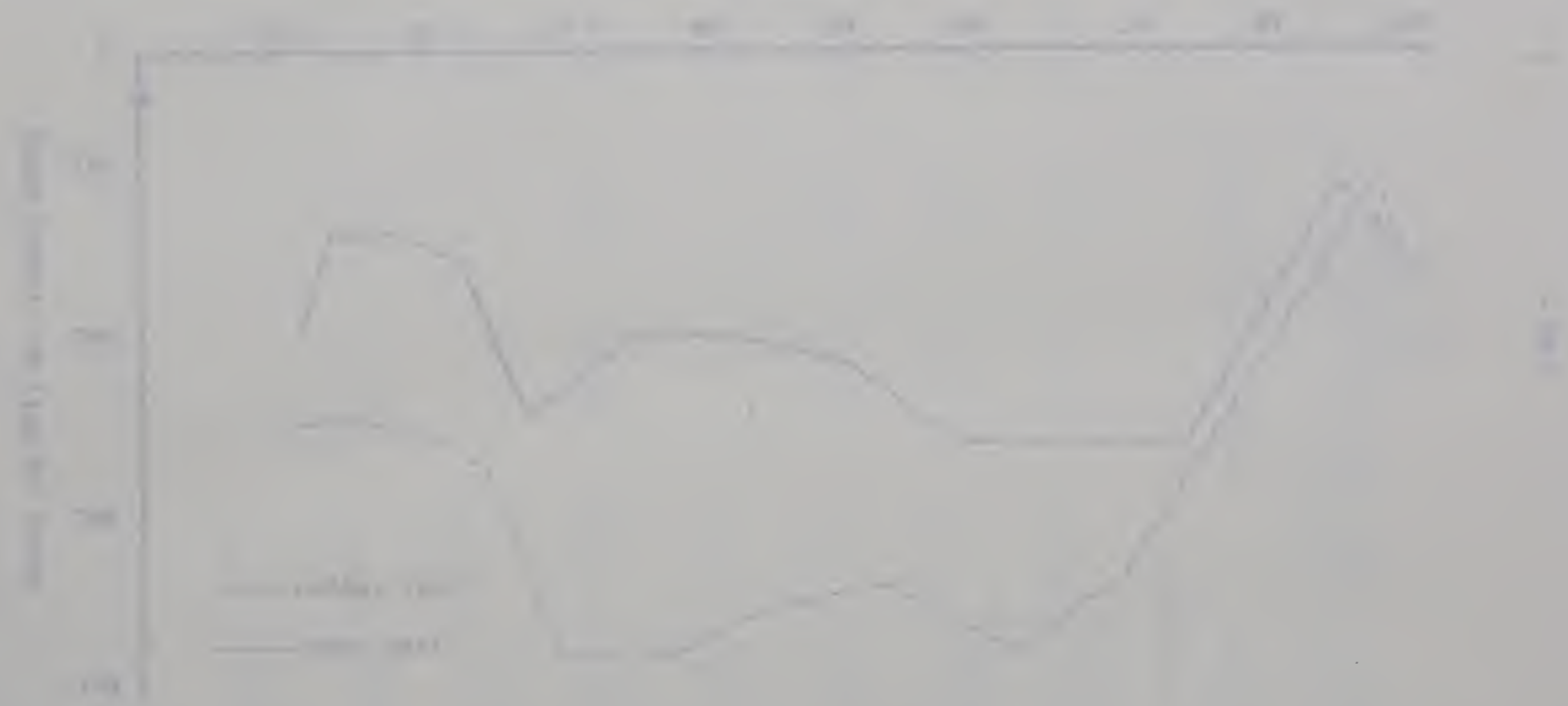
*$P < 0.05$

TABLE 15.

EXPERIMENT 4. MEAN VALUES FOR HEMOGLOBIN.

Experiment no.	4			
Pigs sampled	Final six pigs marketed		All pigs	
Copper supplement	0	+	0	+
Initial period				
No. of pigs	2	3	11	11
Hemoglobin g./100 ml.	11.51	12.16	11.66	12.10
After five weeks				
No. of pigs	3	3	12	12
Hemoglobin g./100 ml.	13.32	12.65	12.07	12.15
At 16 kg.				
No. of pigs	3	3	12	12
Hemoglobin g./100 ml.	12.49	12.50	12.68	12.28
At 23 kg.				
No. of pigs			3	3
Hemoglobin g./100 ml.			12.93	13.32
At 34 kg.				
No. of pigs	3	3	9	8
Hemoglobin g./100 ml.	14.09	13.31	13.60	14.17
At 45 kg.				
No. of pigs			3	3
Hemoglobin g./100 ml.			14.99	13.85
At 57 kg.				
No. of pigs	3	3	6	5
Hemoglobin g./100 ml.	14.78	13.90	14.42	14.30
At 68 kg.				
No. of pigs			3	3
Hemoglobin g./100 ml.			14.39	13.70
At 79 kg.				
No. of pigs	3	3		
Hemoglobin g./100 ml.	14.37	14.29		
At market weight				
No. of pigs	3	3		
Hemoglobin g./100 ml.	13.93	14.34		

Figure 3. Experiment 3. Average values for serum copper.



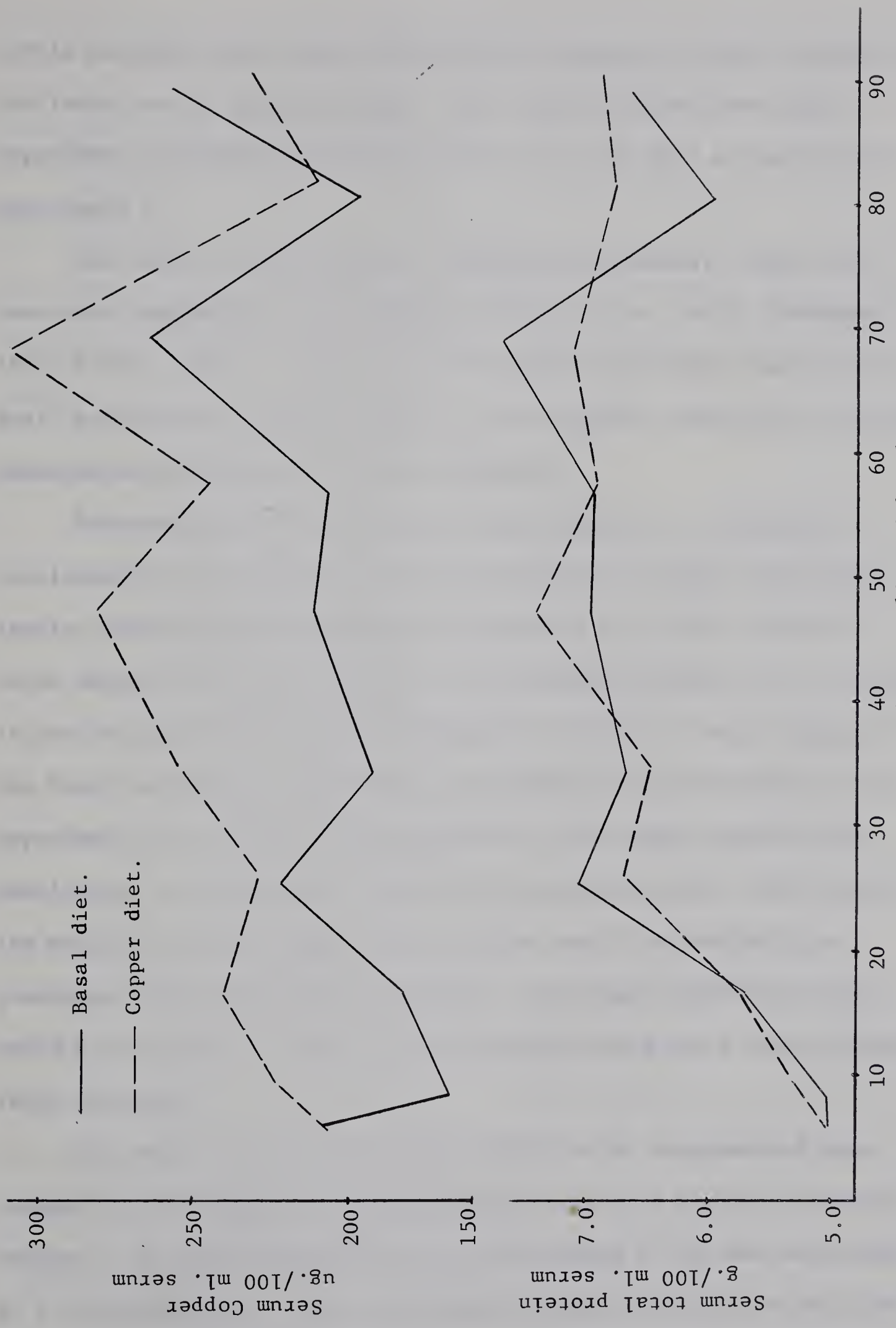


Figure 4. Experiment 4. Average values for serum copper and serum total protein.

levels declined and as pigs reached market weight were quite similar to the levels in the basal fed pigs. The values obtained from pigs in Experiment 3 presented the same patterns for each diet as occurred in Experiment 4.

The levels of serum copper obtained were generally higher than have been reported in the literature (Gubler et al., 1953; Underwood, 1962; Braude, 1965). Difficulties involved in accurately measuring the small quantities of copper present or the different analytical techniques employed could account for these differences.

Interpretation of the plots of serum copper vs. liveweight is complicated as many factors have been reported to affect serum copper levels, although prior information concerning the normal changes in serum copper levels with increase in liveweight of swine is not available. It can be postulated that the difference in levels of serum copper of the basal and copper supplemented pigs during the first weeks of the experiment is a result of the depletion of the copper stores in the basal pigs. The demand for copper by the growing animal could exceed its ability to obtain copper from the diet until its assimilative processes became adequately developed. The copper supplemented pigs would have access to a more readily available form and a higher dietary level of copper.

The reduction of serum copper levels in the supplemented pigs during the final weeks of the experiment could be a result of several factors. The feed intake after all pigs reached 77 kg. was maintained at 2.72 kg./pig/day. Thus, the intake of copper relative to the live-weight of the animal was decreasing. The ability of the animal to remove copper from the blood either by storage or excretion could have

increased and enabled the serum copper to be reduced to a more normal level.

C) Serum total protein concentration.

The average values for serum total protein obtained from basal and copper supplemented pigs in Experiment 4 are represented in Figure 4. There were no marked differences in the two sets of data, which compared favorably with normal values recorded in the literature (Miller et al., 1961a).

D) Serum protein fractions.

The changes in the relative amounts of the albumin, and α- , β- and γ- globulin fractions with growth of the pigs are depicted for both dietary treatments in Figures 5 and 6.

The higher level of serum albumin in the copper-fed pigs during the initial period is emphasized by the ratios of albumin to globulins over this time (Figure 5). The data could be interpreted on the basis of a higher level of albumin synthesis in copper supplemented pigs during this period. Greater variation between basal and copper-fed pigs occurred in the α- and β-fractions. As it was difficult to accurately separate these fractions limited conclusions can be drawn.

In the present study the α-globulin fraction was not consistently increased by copper supplementation. It was reported by Bunch et al., (1965) that ceruloplasmin levels in the blood were not associated with dietary copper levels. Ceruloplasmin, an α-globulin has been reported to contain 58% of the copper in normal pig plasma (Gubler et al., 1953). It is recognized that copper may be ionically bound to serum proteins and transported in this manner rather than as an integral part of protein molecules (Bowland et al., 1961). Consequently it would appear that

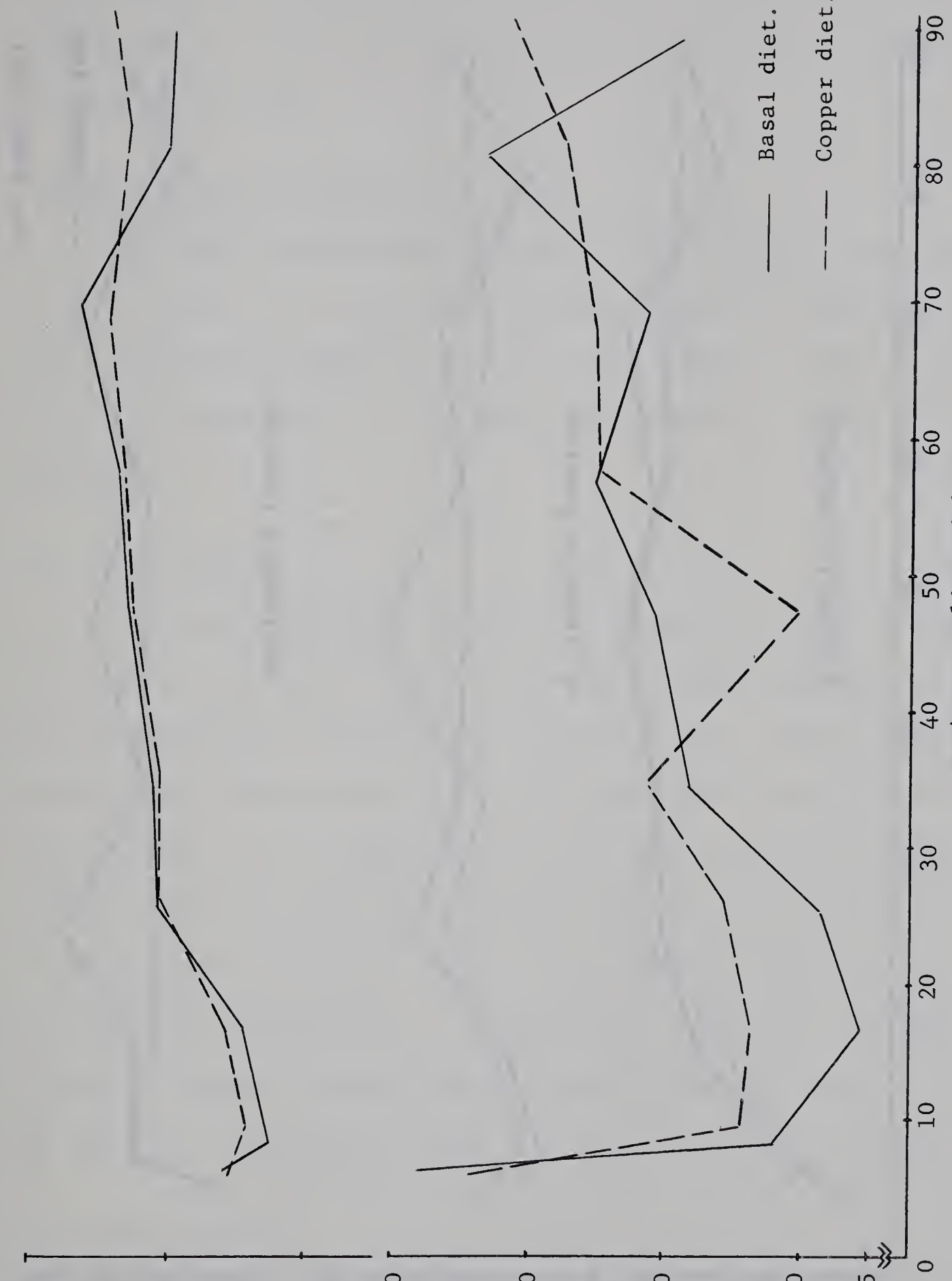
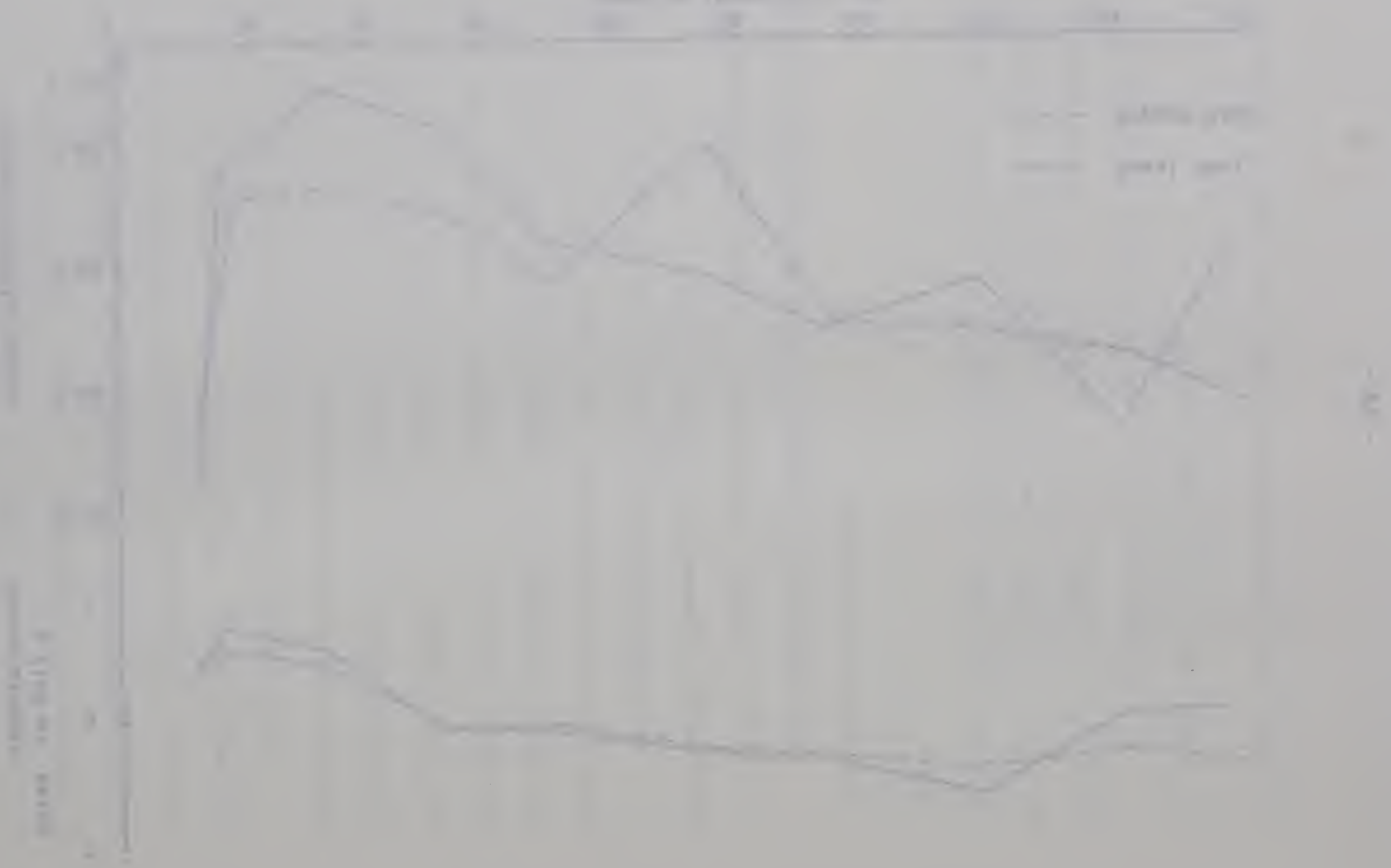


Figure 5. Experiment 4. Average values for serum albumin and albumin/globulin ratios.



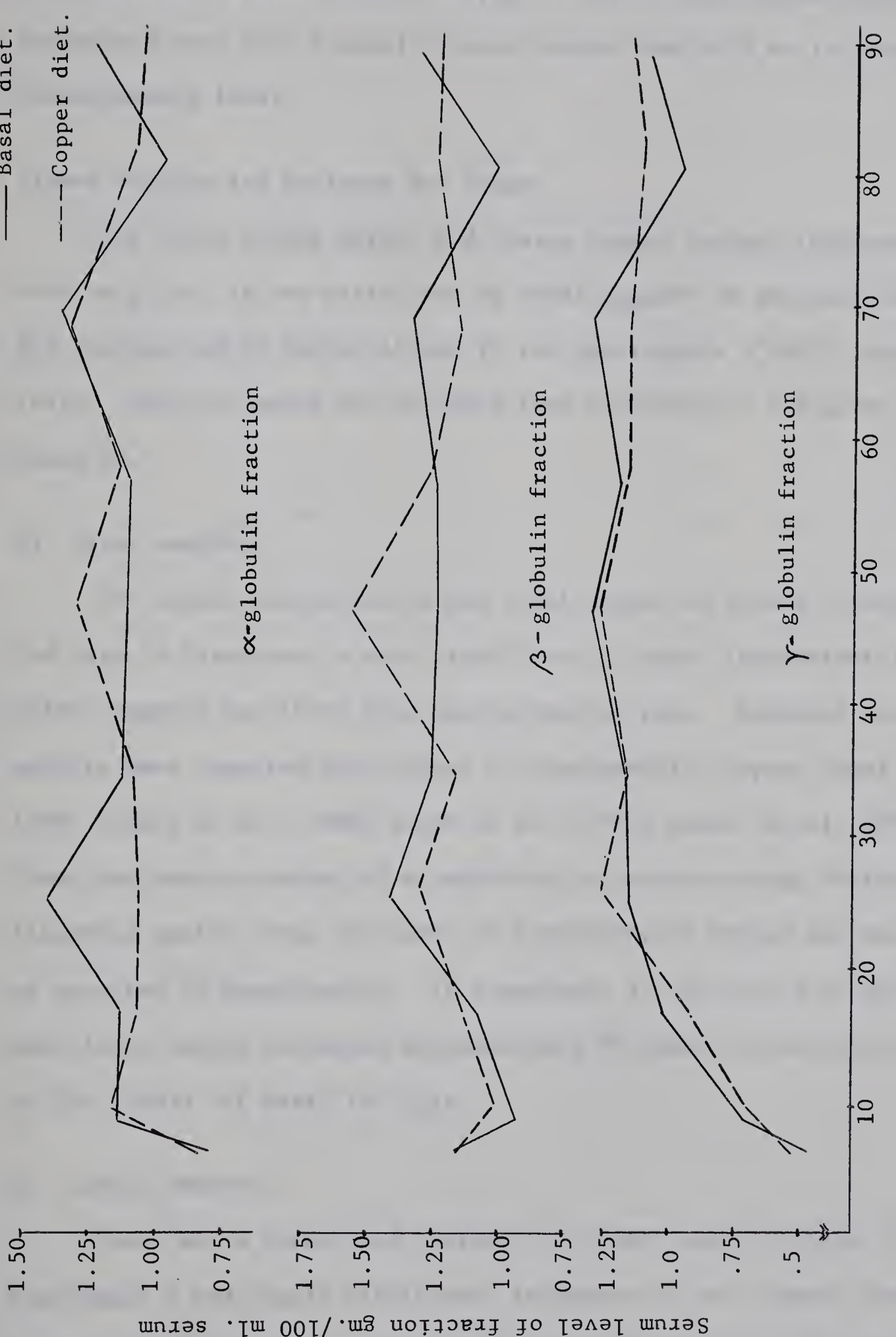


Figure 6. Experiment 4. Average values for α, β and γ-globulin fractions.

the increase in serum copper, in pigs fed the supplemented diet, was associated more with ionically bound copper than with an increase in ceruloplasmin level.

Tissue Weights and Analyses for Copper

The fresh tissue weight and tissue copper content (expressed both as p.p.m. of dry tissue and as total copper) of selected tissues are represented in Tables 16 and 17 for Experiments 3 and 4, respectively. Marginal means for the data from Experiment 4 are given in Table 18.

A) Liver samples.

The copper concentration and total copper in livers of copper-fed pigs in Experiment 4 were significantly higher (approximately eight-fold) compared to livers from unsupplemented pigs. Numerous research workers have reported this effect of supplementary copper (Bass et al., 1956; Ullrey et al., 1960; Bunch et al., 1961; Barber et al, 1961). There has been no report of a reduction in copper storage during the finishing period, when the level of supplementary copper was maintained, as occurred in Experiment 4. In Experiment 3, the livers of pigs fed additional copper contained approximately 30 times the amount of copper in the livers of basal fed pigs.

B) Kidney samples.

There was a three-fold increase in kidney copper levels in Experiment 3 and highly significant increases in both copper concentration and total copper content of kidneys from copper fed pigs in Experiment 4. However, there was a reduction in the kidney copper values in the final period similar to the effect noted for liver copper.

TABLE 16

EXPERIMENT 3. TISSUE WEIGHTS AND COPPER CONTENT.

	Pig Notch	Days on Trial	Final wt. kg. Live	Final wt. kg. Carcass	LIVER Fresh wt. g.	LIVER Copper p.p.m. D.M.	LIVER Copper Total mg.	KIDNEY Fresh wt. g.	KIDNEY Copper p.p.m. D.M.	KIDNEY Copper Total mg.
DIET										
Basal	26	145	92.73	70.45	1226	36.5	13.78	235	35.5	1.65
	37	152	88.86	68.18	1685	23.9	11.68	264	-	-
	75	161	89.77	68.64	1441	28.8	13.57	186	41.7	1.80
	76	161	91.14	69.55	1431	20.3	9.47	233	37.2	1.66
Copper	27	145	88.64	67.73	1377	-	-	218	-	-
	38	138	90.91	65.91	-	-	-	-	-	-
	74	152	91.36	66.36	1517	553.5	274.59	197	130.0	5.67
	77	152	89.09	67.27	1390	998.5	438.54	207	117.4	4.71
Marginal Means										
			Diet	Basal		27.4	12.12		38.1	1.70
				Copper		776.0	356.56		123.7	5.19

TABLE 17
EXPERIMENT 4. TISSUE WEIGHTS AND COPPER CONTENT.

			Pig notch	Days on trial	Final wt., kg. Live	Final wt., kg. Carcass	Liver Fresh wt. g.	Liver Copper p.p.m. D.M.	Liver Copper Total mg.	Kidney Fresh wt. g.	Kidney Copper p.p.m. D.M.	Kidney Copper Total mg.
		Diet										
Slaughter period	0	-	51	0	5.73		186	91.8	4.72	31	50.5	0.34
		-	98	0	5.45		169	77.5	3.70	32	17.9	0.12
		-	104	0	5.86		165	106.1	5.06	42	17.7	0.15
		-	136	0	6.27		175	55.6	2.76	31	19.2	0.12
	1	Basal	69	91	24.77	17.50	721	18.5	3.82	121	39.4	1.02
			85	81	26.14	15.45	689	16.0	3.64	131	39.1	1.11
			134	63	24.77	18.41	633	18.7	3.61	95	35.6	0.77
		Copper	68	60	25.23	17.95	598	102.3	20.01	110	-	-
			87	70	26.36	19.09	636	839.1	153.72	108	123.5	2.96
			135	56	26.36	19.32	687	146.7	30.13	100	87.4	1.88
	2	Basal	56	122	46.59	35.68	1169	20.8	7.14	177	28.5	0.99
			95	110	48.41	36.82	971	23.1	6.84	154	40.9	1.54
			107	105	47.05	35.45	1170	28.1	10.04	137	46.7	1.49
		Copper	55	95	47.50	36.36	1116	33.2	11.85	156	81.0	2.95
			94	117	46.59	34.55	935	169.0	48.97	169	94.3	3.90
			103	88	48.18	37.27	1219	105.9	40.15	166	129.0	4.80
	3	Basal	67	140	69.55	52.73	1125	23.6	8.40	226	47.0	2.11
			86	147	70.00	50.91	1175	22.7	7.91	265	47.3	2.42
			132	139	72.27	52.73	1188	27.1	10.56	177	37.6	1.51
		Copper	60x	147	66.82	50.91	1105	278.6	93.29	231	110.3	4.69
			89[1]	(75)	(23.61)		(617)	(547.2)	(94.56)	(136)	(68.1)	(1.71)
			133	133	69.77	50.91	1227	228.3	82.91	218	81.4	3.51
	4	Basal	57	182	88.64	62.73	1468	30.1	14.66	269	33.7	1.86
			96	175	89.09	67.27	1402	29.8	12.36	221	41.4	1.83
			106	161	89.55	67.73	1485	33.3	14.58	282	53.9	3.31
		Copper	53	182	92.05	69.55	1499	27.9	13.53	268	37.4	2.12
			93	175	89.77	67.73	1385	75.3	32.21	246	30.6	1.61
			109	168	90.68	66.36	1410	237.3	108.06	228	83.9	3.95

[1]Sacrificed.

Table 17 continued.

		Heart			Spleen					
			Copper			Copper				
		Fresh wt. g.	p.p.m. D.M.	Total µg.	Fresh wt. g.	p.p.m. D.M.	Total µg.	Pituitary fresh wt.mg.	Muscle copper p.p.m. D.M.	Hair copper p.p.m. D.M.
	Diet									
Slaughter period	0 -	31	16.1	113	12	5.1	13	41.6	-	14.3
	-	33	13.3	100	13	5.8	16	48.5	3.9	11.8
	-	49	15.1	158	10	4.6	10	53.5	5.3	9.8
	-	32	14.9	107	14	5.5	17	49.2	5.1	11.1
	1 Basal	95	17.1	335	40	4.5	40	144.4	3.1	17.2
		108	18.0	381	45	4.3	43	166.8	4.5	13.4
		109	16.5	370	40	3.9	35	151.9	3.5	10.4
	Copper	120	15.5	339	32	5.3	37	149.0	3.6	14.9
		135	16.9	429	43	4.7	48	102.0	4.3	49.8
		115	15.6	360	37	4.4	35	144.9	2.9	23.8
	2 Basal	164	14.4	508	73	8.1	140	199.5	-	10.3
		182	15.2	575	63	5.4	79	217.7	-	11.1
		183	14.7	560	73	4.9	82	204.5	3.0	6.7
	Copper	154	17.0	583	55	6.7	81	183.8	3.2	19.3
		166	14.3	533	55	5.2	77	164.1	4.2	33.4
		173	16.1	580	75	5.2	89	237.6	3.0	29.5
	3 Basal	231	16.5	851	92	6.3	131	265.1	2.7	11.9
		246	15.8	891	112	5.0	121	238.6	3.1	-
		219	14.5	748	81	5.4	97	313.5	2.2	6.6
	Copper	182	16.0	696	88	6.4	124	222.6	1.8	-
		(116)	(17.2)	(399)	(37)	(5.5)	(44)	(137.5)	(7.1)	(46.6)
		212	15.2	742	97	12.0	260	275.3	2.3	28.3
	4 Basal	257	15.5	947	130	5.8	165	278.0	2.3	9.5
		270	14.7	969	103	7.9	184	248.0	2.0	9.4
		360	14.0	1152	128	6.5	174	312.8	3.1	7.6
	Copper	231	14.9	857	111	6.3	156	297.5	2.2	18.0
		259	14.9	879	89	5.2	102	298.7	3.0	18.4
		294	12.1	894	107	7.6	179	295.7	1.9	19.6

TABLE 18

EXPERIMENT 4. MARGINAL MEANS OF TISSUE WEIGHTS AND COPPER CONTENT.

	Liver Copper		Kidney Copper		Heart Copper		Spleen Copper	
	p.p.m. D.M.	Total mg.	p.p.m. D.M.	Total mg.	p.p.m. D.M.	Total ug.	p.p.m. D.M.	Total ug.
Diet								
Basal	24.32	8.63	40.92	1.66	15.57	690.58	5.67	107.58
Copper	208.08*	60.24**	88.33**	3.24**	15.34	634.25*	6.52	115.00
Period								
0	82.75	4.06	26.32	0.18	14.85	119.50	5.25	14.00
1	190.22	35.82	71.73	1.69	16.60	369.00	4.52	39.67
2	63.35	20.83	70.07	2.61	15.28	556.50	5.91	91.33
3	138.95	48.53	69.90	3.05	15.60	774.50	7.38	154.17
4	72.28	32.57	46.82	2.45	14.35	949.67	6.55	160.00

	Muscle Copper	Hair Copper	Heart Weight	Spleen Weight
	p.p.m. D.M.	p.p.m. D.M.	g.	g.
Diet				
Basal	2.96	10.27	202.0	81.7
Copper	2.87	25.97**	186.5	73.4
Period				
0	4.77	11.75	36.2	12.2
1	3.65	21.58	113.7	39.5
2	3.23	18.38	170.3	65.7
3	2.35	18.77	214.5	93.7
4	2.42	13.75	278.5	111.3

*Significant at $P < 0.05$.

**Significant at $P < 0.01$.

The results were similar to previous reports (Barber et al., 1957). Both the liver and kidney are regarded as natural copper storage tissues.

C) Heart samples.

The total copper content in the hearts of copper fed pigs was significantly reduced compared to hearts from the basal pigs. The concentration of copper was similar for both groups but the fresh weight of the hearts from copper fed pigs were on the average 7% less than those from basal fed pigs. There are few reports on the effect of supplementary copper on heart copper levels, although, Barber et al. (1961) found a reduction in the copper concentration and an indication that a level of 0.1% copper sulfate reduced heart weight and increased organ moisture content.

D) Spleen samples.

Copper supplementation of the diet in Experiment 4 did not appear to affect the copper levels in the spleen. The levels of copper were comparable to those reported by Barber et al. (1961).

E) Muscle samples.

Copper fed pigs had a lower average level of copper in the muscle sample than basal pigs; the difference was not significant. Over the experimental period copper concentration in the samples declined from an average of 4.8 p.p.m. to 2.4 p.p.m. on a D.M. basis. Investigating the effect of feeding 250 p.p.m. of supplementary copper on the copper concentration of the longissimus dorsi muscle, Taylor and Thomke (1964) found no difference between samples from basal and copper fed pigs. In contrast, Bunch et al. (1961) reported an increase from 5 p.p.m. to 16.0 p.p.m. D.M. in the copper concentration with supplementary copper. Barber et al (1961) reported that the copper concentration of the psoas muscle increased from 3.0 p.p.m. to 5.0 p.p.m. D.M. when the

diet was supplemented with 250 p.p.m. of copper.

F) Pituitary.

No effect was observed with respect to the weights of the pituitaries obtained from basal or copper fed pigs.

G) Hair samples.

The copper concentration in hair was significantly higher for copper supplemented pigs. Black hair contained a higher level of copper (54.4 p.p.m.) than white hair (21.3 p.p.m.) from the same animal. However, in hair samples used for comparative analyses only white hair was used. Therefore, hair color is not the explanation for the differences observed. There are no published reports of hair analyses on copper supplemented pigs.

GENERAL DISCUSSION

Braude (1965) discussing the results of a large co-ordinated trial stated, "It is apparent...that the recorded variation in response to copper was random and obviously due to other factors operating when complex biological phenomena are involved...it becomes clear how misleading it can be to draw conclusions from a single test, however well conducted."

The experiments which formed the basis of the present study were similar in the variety of responses observed to those reported in the literature. With regard to the variations encountered the results can be discussed only as indicative of trends rather than presenting conclusive evidence for proposed metabolic roles of supplementary copper.

The predominant effect during the experiments was an improvement in rate of gain and efficiency of feed utilization during the rearing period. Several research workers have reported a similar effect, notably Lucas, Livingstone and McDonald (1961); Lucas, Livingstone and Boyne (1962). The lack of response during the finishing period in some reports could, in part, be a result of reduction of the dietary protein level, a normal practice in formulation of finishing diets.

It has been suggested that the action of supplementary copper could be a result of its effect upon the gut flora (Hawbaker et al., 1961). It is difficult to correlate this mechanism, which presumably would be operative over the entire growing period, with a benefit which in many cases is restricted to the early part of the growing period.

The initial observations of Braude (1945) who reported that pigs exhibited a definite craving for copper could suggest that the pigs were

reacting against an imposed copper deficiency. The trend towards intensive pork production with sows tending to produce more pigs per litter and having less access to pasture could result in a reduction of copper stores in the new born. The reduced reserve of copper coupled with increased demand as a result of faster growth rates could result in a temporary deficiency of copper until the digestive system of the pig was sufficiently developed.

Comparing the data on growth measurements and serum analyses for basal and copper fed pigs during the rearing period, the differences suggest that the basal fed pigs were not obtaining sufficient copper to meet the requirement at that stage. A reduction in activity of cytochrome oxidase has been reported in the heart muscle of deficient swine (Lemberg, Newton and Clarke, 1962). In rats reduction in cytochrome oxidase activity was one of the first symptoms of copper deficiency (Gallagher, Judah and Rees, 1956a). It could be expected that reduction in activity of cytochrome oxidase would be reflected in a reduced efficiency of nutrient utilization and/or a slower formation of body tissue.

The serum copper levels of basal fed pigs declined over the first few weeks of the rearing period; this could be explained by an increase in copper utilization, excretion or storage or by decreased assimilation from the feed or a combined effect. The serum levels of copper fed pigs exhibited no such decline suggesting that there was increased assimilation of copper sufficient to meet or surpass the requirement at that time.

The mechanism of absorption of copper is not fully understood. Saltman, Alex and McCormack (1959) favor a passive rather than active

transport mechanism. The composition of the diet has been shown to affect copper availability and it has been reported that only 2 to 10 per cent of ingested copper is absorbed by the pig (Bowland et al., 1961). The pigs ability to excrete copper is apparently limited (Mahoney et al., 1955). This could result in a rate of assimilation of copper from the diet which was adequate during the rearing period, when requirement for copper was high, but which was excessive during later stages of growth, when the requirement was reduced. The data on hemoglobin values suggest that the copper retention in supplemented pigs was not so great as to adversely affect hematopoesis. However, the increased copper retention may have had an adverse effect upon gain and feed conversion during the finishing period for the copper fed pigs.

The incidence of a stiff leg condition in three littermates (#85 on basal feed, #87 and #89 on copper feed) bore some resemblance to symptoms of copper deficiency (Teague and Carpenter, 1951). However, upon analysis of the tissues from #89 there was little difference in copper levels from other pigs on the same treatment (Table 17). No explanation apart from the official autopsy report of septic arthritis can be offered.

The copper concentrations in tissues, other than the liver and kidney, suggest that in pigs fed no supplementary copper, growth normally results in an increase in total copper content which can almost wholely be accounted for by the increase in size of the tissue. Supplemented pigs exhibited a similar pattern except for the copper concentrations in hair which appeared to be related to the serum copper levels.

GENERAL SUMMARY AND CONCLUSIONS

Responses of pigs to the inclusion of 0.1 per cent copper sulfate in the feed have been studied under a variety of dietary conditions over the range from approximately 5.0 to 92.0 kg. liveweight. Apparent trends often did not reach a level of statistical significance on account of a high degree of within treatment variation. While the responses to copper could be related to some extent to the method of feeding or to the level or source of dietary protein, the major benefits from copper supplements occurred during the initial 6 weeks of all experiments conducted. During this period improved rate of gain and efficiency of feed conversion were observed.

Results from digestibility trials during the rearing period suggested that copper supplemented pigs were utilizing their diet more efficiently than basal fed pigs. It is recognized that a small advantage in the early stages of growth could be magnified throughout the experimental period by either method of feeding. Faster growing pigs would have consumed more feed under ad libitum conditions and would have been allocated more in experiments where feed was restricted to a scale based on liveweight. Some individual pigs did follow this trend but in general the advantages of feeding supplementary copper tended to be less apparent as the pigs gained weight.

The blood and tissue analyses show that individual animals have a relatively similar pattern of internal distribution of copper but exhibit widely different tissue levels. It was not determined whether these differences were a result of enhanced absorption, greater utilization or impaired ability to excrete copper. The blood hemoglobin values did not reflect an adverse or beneficial response to the higher dietary copper levels.

The serum copper values tended to be similar for all pigs at the beginning and termination of an experiment. During the growth period pigs fed copper had a consistently higher serum copper level than unsupplemented pigs. It could be suggested from the shape of the serum copper curves that basal fed pigs might be obtaining an inadequate amount of copper from the diet in the initial period which could be responsible for the growth check, compared to copper fed pigs. Alternatively the supplemented pigs might be absorbing an excess over their requirements which could have been reflected in the lowered rates of gain in the final period.

Serum total protein and protein fractions showed little variation between pigs fed supplemented or unsupplemented diets. The values were comparable to accepted normal levels.

The tissue copper levels, except in the liver and kidney, tended to be maintained or gradually declined over the experimental period. Supplementary copper increased liver, kidney and hair copper content and appeared to reduce heart weight.

The carcass measurements which were indicators of leanness or fatness in the carcasses were generally improved for pigs which had been fed copper although ad libitum fed pigs which had received the fishmeal based ration with copper had noticeably softer carcass fat which resulted in lower carcass grades.

From the results of the experiments it is suggested that under certain conditions individual pigs may be subjected to a limited copper deficiency in the post-weaning period. Supplementation with copper appears to be of value at this stage of growth up to approximately 50 kg. liveweight. If copper is continuously supplied to market weight it should be included at a level which would not lead to excessive tissue accumulation.

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APPENDIX A

Determination of copper with zinc dibenzyldithiocarbamate.

Based on the method of Andrus (1955).

Materials and Method.

Glassware -- All glassware was thoroughly cleaned with hot detergent solution, rinsed and soaked overnight in 1:1 nitric acid. After soaking it was rinsed with hot tap water, distilled water and demineralized water, then dried at 100-110°C. Before use the glassware was stored separately from other containers and protected from contamination.

Water -- only demineralized water was used. Distilled water was passed through a Bantam Demineralizer[1] equipped with a standard cartridge.

Reagents.

1) Sulfuric acid, concentrated 95.0 - 98.0 per cent H_2SO_4. Micro analytical purity.
2) Nitric acid, concentrated sp. gr. 1.416-1.426. Reagent grade.
3) Perchloric acid, 70-72% $HClO_4$. Reagent grade.
4) Digestion mixture H_2SO_4: $HClO_4$: HNO_3 1.5:1:7.5.
   To 1500 ml. concentrated nitric acid slowly add 200 ml. of perchloric acid in small volumes. Cool and carefully add 300 ml. concentrated sulfuric acid in small volumes, allowing the mixture to cool after each increment of sulfuric acid.
5) Hydrogen peroxide, 100 volume 30%. Baker Analyzed Reagent.
6) Carbon tetrachloride. Spectrophotometric quality.

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[1]Barnstead Still and Sterilizer Co., Boston 31, Mass.

7) Zinc dibenzyldithiocarbamate. B.D.H. Laboratory Reagent.

8) Carbamate reagent. A 0.01 per cent (w/v) solution of zinc dibenzyldithiocarbamate in carbon tetrachloride.

9) Standard copper solution 0.2 mg./ml. Dissolve approximately 0.2 gm. metallic copper, reagent grade, in 10 ml. concentrated HNO_3. Heat till no brown fumes are produced and solution clears. Transfer to 1000 ml. volumetric flask, add 10 ml. concentrated H_2SO_4 and make up to volume with water. Required dilutions are made daily.

Procedure.

1) Weigh out on filter paper not more than 1 gm. or 0.5 gm. of air dry feed or fecal sample, respectively. For dried tissue samples the weight of sample used is adjusted according to the expected copper content, i.e. approximately 0.3 gm. of dried liver, 0.5 gm. of dried kidney, 0.7 gm. of dried heart or spleen and 1.0 gm. of dried muscle were used.

2) Place each sample in a 100 ml. kjeldahl flask, graduated at 100 ml. and after addition of 10.0 ml. of digestion mixture allow to stand overnight.

3) Include a blank determination consisting of filter paper and digestion mixture and standard solutions with each set of unknown.

4) Heat the flasks, cautiously in the initial stages, on a microburner until the solution clears and then cool.

5) Add 1.0 ml. hydrogen peroxide, heat to appearance of white fumes and cool.

6) Add 5.0 ml. water, reheat to appearance of white fumes and cool.

7) For flasks containing less than 25 ug. total copper transfer contents directly to separating funnels graduated at 25.0 ml. and make up to volume. Fill remaining flasks with water up to 100 ml. and transfer aliquots ($\leq$ 25 ml.) containing not more than 25 ug. copper to separating funnels. Add sufficient concentrated H_2SO_4 to bring total content* of H_2SO_4 up to 1.25 ml. before diluting to final volume of 25 ml. with water.

8) Add 10.0 ml. carbamate reagent and shake for one minute.

9) Draw off approximately 5 ml. of the CCl_4 layer through absorbent cotton directly into spectrophotometric tubes and read absorbances at 435 mu. against CCl_4. Color is stable for at least an hour but it is advisable to read as soon as possible to avoid effect caused by evaporation of the CCl_4.

Discussion.

The method of Martens and Githens (1952) is basically the same as the method of Andrus (1955). The first mentioned authors claim that the carbamate solution never gave any evidence of instability in contact with 1 N sulfuric acid from which the extraction was normally made. However, it is difficult to correlate the last statement with the details of their procedure which if followed could result in solutions equivalent in normality of sulfuric acid from 1.8 N to less than 1.0 N. The method of Andrus (1955) results in a more constant level of acidity provided aliquots of the final digest are not used.

Initially the method of Martens and Githens (1952) was employed.

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*It is assumed that approximately 1.25 ml. of the initial 1.5 ml. H_2SO_4 remains after digestion of the sample.

Difficulties were encountered in obtaining a linear standard curve. These difficulties were attributed to a possible pH effect upon the formation of the copper-carbamate complex. Consequently this was studied, the results are shown in Figures 7 and 8. The effect of acidity of the solution upon the absorbance is apparent and presents an obvious difficulty as the pH or content of H_2SO_4 of the digested sample is not determined. For the present analyses it was decided to attempt to maintain a relatively constant content of sulfuric acid in the final solution from which the extraction was made. An assumption was made in that a final level of 1.25 ml. of concentrated sulfuric acid was set, this was 0.25 ml. lower than the initial level,which would presumably be reduced to a slight extent during the digestion procedure. A check with short range Alkacid[1] paper suggested that a final level of 1.25 ml. concentrated sulfuric acid was a valid assumption to make Consequently, amounts of concentrated sulfuric acid were added when necessary to bring the total content up to 1.25 ml. prior to addition of the carbamate reagent.

A further difficulty was encountered during the final sample analyses. It appeared that a 0.01% level of zinc dibenzyldithiocarbamate was insufficient to fully complex the copper present in the final digests. The level of zinc dibenzyldithiocarbamate was then raised to 0.02%. Subsequent analyses conducted using the adjusted carbamate reagent were performed adequately. It can be surmised that changes, possibly a slow degradation, could occur in the zinc dibenzyldithiocarbamate with time and thus account for a reduction in complexing ability.

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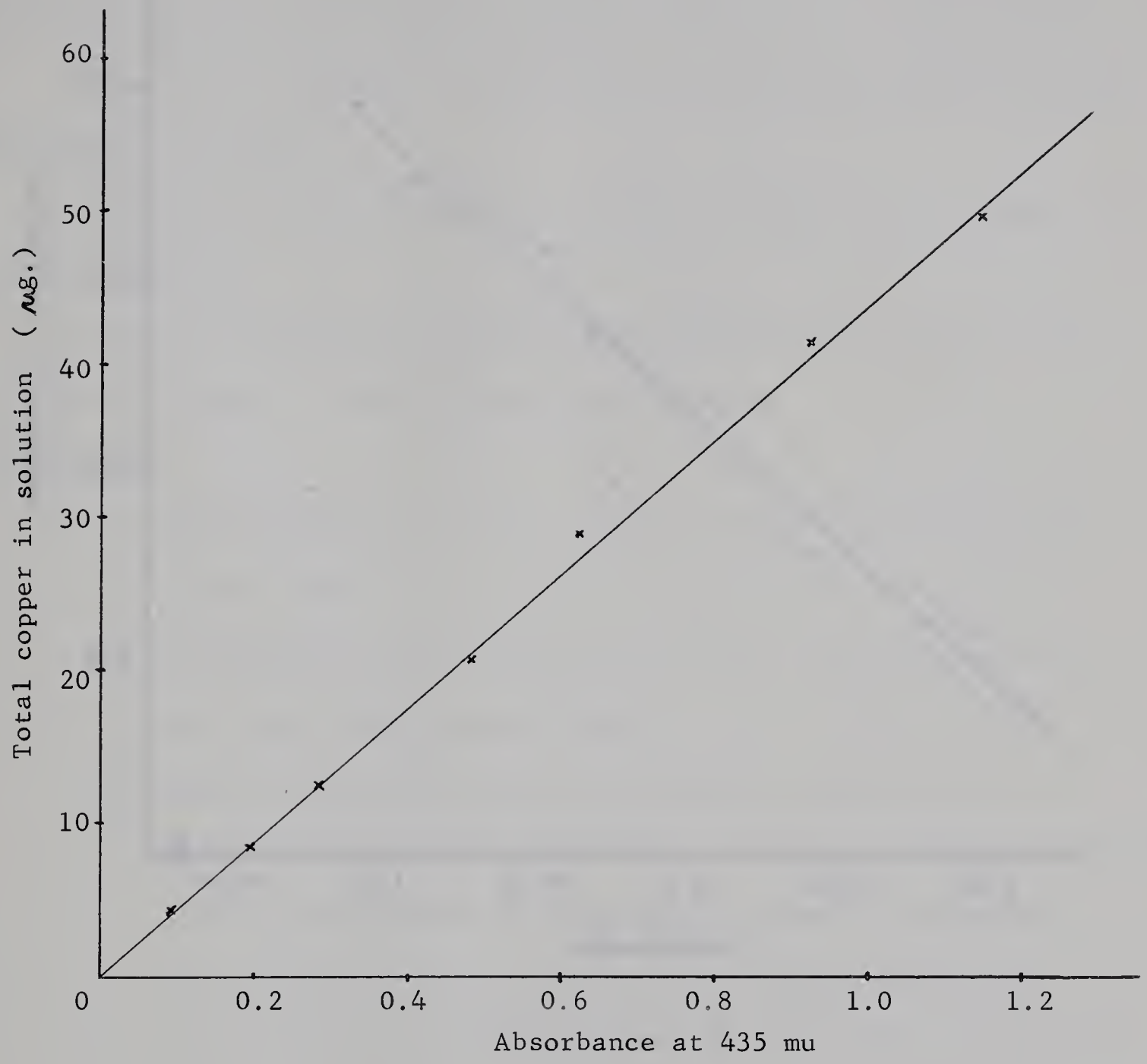
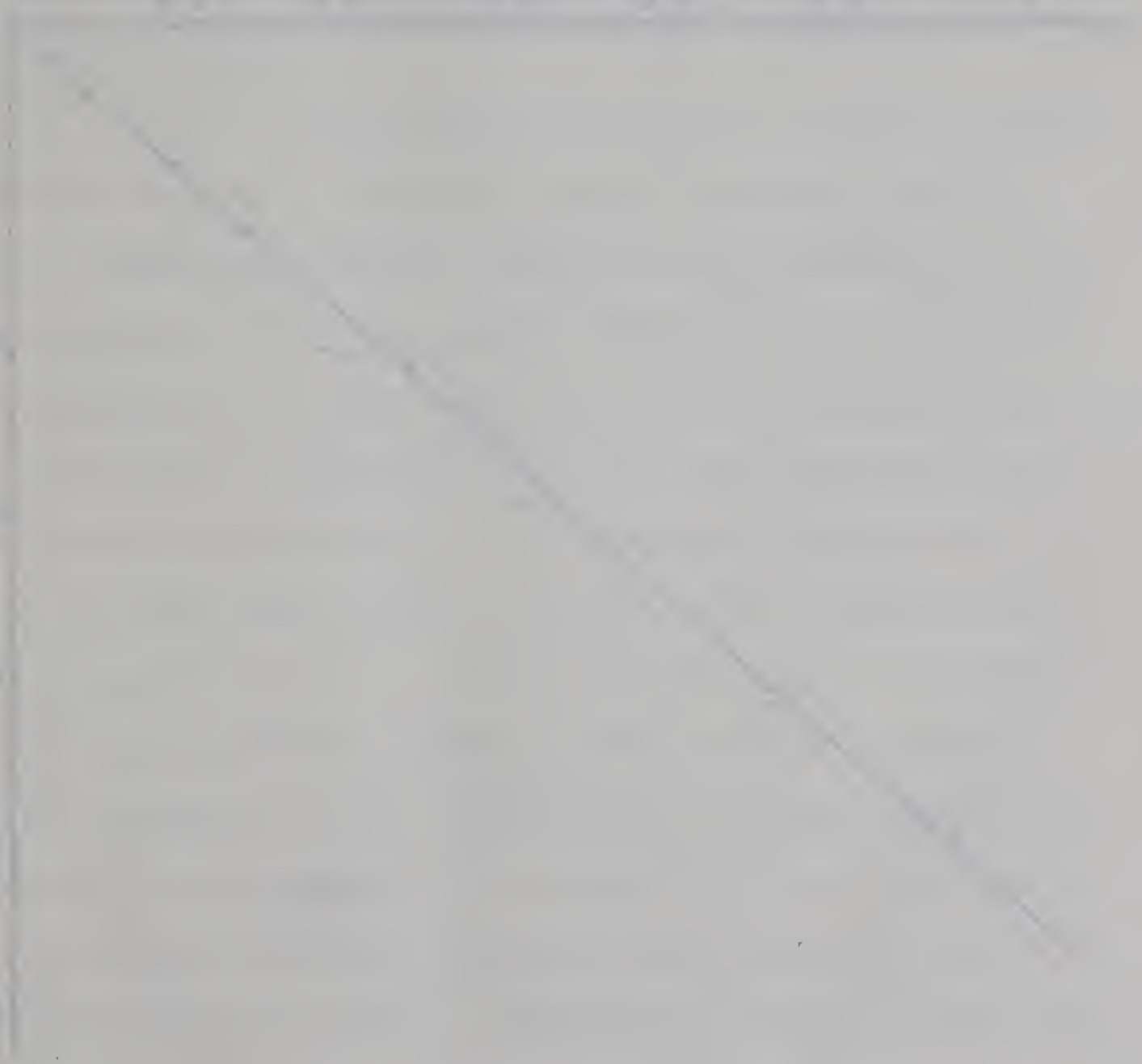
[1]Fisher Scientific Co. Fair Lawn, N.J.



Copper standards from 0 - 50 ug., extracted from 1 N sulfuric acid solutions with 0.01% zinc dibenzyldithiocarbamate in carbon tetrachloride.

Figure 7. Standard curve for copper determination.



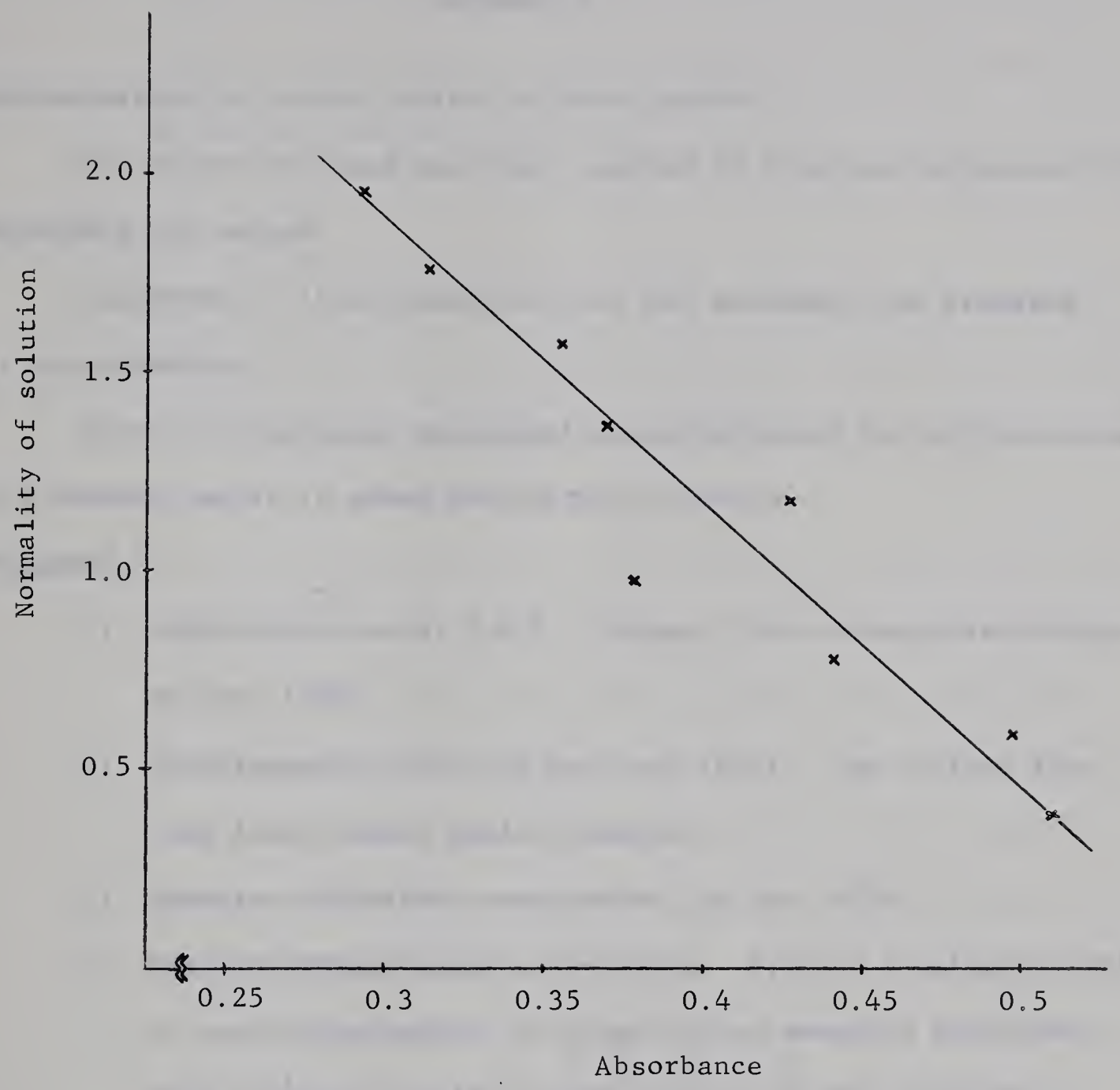


Figure 8. Absorbance of the complexes formed by zinc dibenzyldithiocarbamate and 20 u'g. of copper when extracted from solutions of different normalities.

APPENDIX B

Determination of copper content of hair samples

The method followed was that reported by Rice and Goldstein (1961).

Materials and method.

Glassware -- It is essential that all glassware be prepared as in Appendix A.

Water -- Use resin deionized[1] distilled water for all solutions and whenever water is added during the procedure.

Reagents.[2]

1) Hydrochloric acid, 2.0 N. Prepare from concentrated reagent sp. gr. 1.19.
2) Trichloracetic acid, 20 per cent (w/v). Use sulfate free, iron free highest purity chemical.
3) Ammonium hydroxide concentrated, sp. gr. 0.90.
4) Oxalyldihydrazideammonia solution. Prepare a saturated solution of oxalyldihydrazide, in concentrated ammonium hydroxide. This solution should be used only on the day prepared.
5) Acetaldehyde aqueous solution, 50 per cent (v/v). Store this reagent in a refrigerator.
6) Ethylene diamine tetracetic acid, disodium salt (EDTA).
7) Citric acid, crystal.
8) Standard copper solution. Dissolve 50 mg. reagent grade metal in a 5.0 ml. concentrated nitric acid by warming gently. When dissolved, heat cautiously till clear blue solution obtained. Transfer quantitatively to 1000 ml. volumetric

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[1]See Appendix A.

[2]Certified reagent grade is used where quality not specified.

flask, add 5.0 ml. concentrated sulfuric acid and dilute to 1000 ml. with water. Prepare dilutions to give a range of standards from 1 to 6 ug./ml.

Procedure.

1) Weight accurately into 13 by 100 mm. test tube 40 to 80 mg. of dry hair. Carry two empty tubes for blank and additional tubes equivalent to number of standards to be employed. At least three standards should be included.
2) To all tubes add 1.4 ml. 2.0 N hydrochloric acid and 2.0 ml. 20% trichloracetic acid. Mix with the aid of a Vortex[1] or similar mixer, cover tubes with aluminum foil and place in aluminum test tube rack.
3) Place rack in boiling water bath, with the tubes immersed to the level of the contained solution, for 15 minutes. Allow to cool at room temperature.
4) To tubes containing hair add 1.0 ml. water, to blank tubes containing acid mixtures add 1.0 ml. water, for standard tubes add 1.0 ml. appropriate standard solution, mix thoroughly.
5) To dry clean spectrophotometric tubes containing 5-10 small crystals of citric acid add accurately 2.0 ml. of hair, blank or standard solutions. Mix to dissolve the citric acid.
6) To one of the blank tubes add a pinch of EDTA powder and mix to dissolve.
7) In succession, add and mix 0.50 ml. oxalyldihydrazideammonia

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[1]Scientific Industries Inc., Queens Village 29, New York.

solution and 0.50 ml. cold acetaldehyde aqueous solution. Allow to stand for 30 minutes at room temperature.

8) Determine absorbance of solutions at 545 mu against the blank tube containing EDTA. The color is stable for at least 30 minutes.

9) Calculate the copper content from a standard curve constructed from the standard tube values.

APPENDIX C

Determination of serum copper with oxalyldihydrazide.

The basis of the method is that of Rice (1960).

Materials and method.

Glassware -- Essential that all glassware be prepared as outlined in Appendix A.

Water[1] -- Demineralized water is used for all solutions.

Reagents[2].

1) Oxalyldihydrazide, Eastman reagent grade.
2) Hydrochloric acid, 2.0 N, containing 0.10 per cent (w/v) oxalyldihydrazide. Prepare from concentrated reagent, sp. gr. 1.19.
3) Trichloracetic acid, 20.0 per cent (w/v). Use sulfate free, iron free, highest purity chemical.
4) Ammonium hydroxide, concentrated, sp. gr. 0.90.
5) Acetaldehyde aqueous solution, 50 per cent (v/v). Store this reagent in the refrigerator.
6) Ethylene diamine tetraacetic acid disodium salt (EDTA).
7) Citric acid crystal.
8) Standard copper solution 60 ug./ml. Dissolve 60 mg. metallic copper in 6.0 ml. concentrated nitric acid by warming gently. Heat cautiously to drive off brown fumes until a clear blue solution obtained. Transfer quantitatively to 1000 ml. volumetric flask and, after adding 5.0 ml. concentrated H_2SO_4 make up to volume with water. Prepare dilutions to give a range of standards from 0.5 to 6.0 ug. copper/ml.

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[1] See Appendix A.

[2] Certified reagent grade is used where quality not specified.

Procedure.

1) Into a 10 by 75 mm. test tube put 1.0 ml. of serum and 1.0 ml. 2.0 N hydrochloric acid which contains 0.1 per cent oxalyl-dihydrazide.
2) For each set of unknowns carry two water blanks and at least 1 standard tube (2 ug. copper/ml.).
3) Add 1.5 ml. 20 per cent trichloracetic acid, mix well, close the tube with a clean dry stopper, let stand at room temperature for five minutes and centrifuge rapidly (approximately 2000 r.p.m.) for 15 minutes.
4) Into spectrophotometric tubes, which contain 5-10 small crystals of citric acid, 2.0 ml. of the clear supernatant from each blank, standard and unknown are accurately transferred.[1] Agitate to dissolve the crystals.
5) To only one of the blanks add a pinch of EDTA and mix.
6) In all tubes, mix 0.50 ml. ammonium hydroxide and 0.50 ml. cold acetaldehyde solution in that order.
7) Allow tubes to remain at room temperature for 30 minutes, then determine the absorbance at a wave length of 545 mu against the blank containing the EDTA. The color is stable for at least 30 minutes.
8) Construct standard curve and calculate copper content in serum directly.

The major modification of the original method was in using 1.0 ml. of the hydrochloric and 1.5 ml. of the trichloracetic acid reagents rather the

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[1] A 2.0 ml. insulin syringe (Long type - Becton, Dickinson and Co., Rutherford, N.J.) was employed. Its accuracy was checked by weighing an indicated 2.0 ml. volume of water and calculating the equivalent volume.

published values of 0.7 and 1.0 ml., respectively. It was found with the original volumes that there was insufficient total volume (2.7 ml.) from which to remove a 2.0 ml. aliquot.